National Library of Canada
Canadian Theses Service
Ottawa, Canada
K1A 0N4

Bibliothèque nationale du Canada
Service des thèses canadiennes

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30, and subsequent amendments.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30, et ses amendements subséquents.

NL-339 (r. 88/04) c

Canada

UNIVERSITY OF ALBERTA

THE ROLE OF GRANULAR ACTIVATED CARBON AS A SUPPORT MEDIUM IN THE ANAEROBIC DEGRADATION OF PHENOL

By

© STEPHEN A. CRAIK

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS OF THE DEGREE

OF MASTER OF SCIENCE

DEPARTMENT OF CHEMICAL ENGINEERING

EDMONTON, ALBERTA

SPRING 1990

National Library of Canada
Bibliothèque nationale du Canada

Canadian Theses Service
Service des thèses canadiennes

Ottawa, Canada
K1A 0N4

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30, and subsequent amendments.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30, et ses amendements subséquents.

ISBN 0-315-60283-X

NL-339 (r. 88/04) c

Canada

micro.

UNIVERSITY OF ALBERTA

RELEASE FORM

NAME OF AUTHOR: Stephen A. Craik

TITLE OF THESIS: The Role of Activated Carbon as Support Medium in the Anaerobic Degradation of Phenol

DEGREE: Master of Science

YEAR THIS DEGREE GRANTED: 1990

Permission is hereby granted to the UNIVERSITY OF ALBERTA LIBRARY to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly or scientific research purposes only.

The author reserves other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.

SIGNED: Steve Craik

PERMANENT ADDRESS:

Apt. 8, 10636–85 Avenue
Edmonton, Alberta
T6E 2K7

Date: March 9, 1990

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled THE ROLE OF GRANULAR ACTIVATED CARBON AS A SUPPORT MEDIUM IN THE ANAEROBIC DEGRADATION OF PHENOL submitted by STEPHEN A. CRAIK in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE.

M.R. Gray (Supervisor)

R.E. Hayes

P.M. Fedorak

S.E. Hrudey

Date: March 9, 1990

ABSTRACT

An experimental program was undertaken with the objective of determining the role that granular activated carbon (GAC) plays in enhancing the anaerobic bio–treatment of a phenolic wastewater, apart from serving as a physical adsorbent. GAC was employed as a biological support medium in a laboratory–scale, experimental bioreactor, treating a model wastewater containing inorganic nutrients and phenol as the major carbon source. The bioreactor was operated at pseudo–steady state in order to evaluate the phenol–degrading kinetics of a culture attached to GAC particles maintained in an expanded–bed column. The attached culture did not exhibit superior phenol–degrading activity over non–attached culture forms. The specific phenol–degrading activities of the attached, flocculated and suspended cultures were all in the range of 0.14–0.19 mg phenol/mg VSS/d.

Serum bottle cultures containing GAC–supported and flocculated biomass from the reactor were subject to nominal shock loads of phenol concentration in the supernatant of 1000, 1500 and 2000 mg/L. Above 1500 mg/L both types of culture experienced essentially total inhibition of phenol–degrading activity. Below 1200 mg/L, the activity of the GAC–supported culture was not inhibited whereas that of the flocculated culture was reduced significantly. Prevention of feedback inhibition of phenol degradation by adsorption of the metabolic intermediate, acetic acid, was proposed as the mechanism which accounted for the superior performance of the GAC culture.

Of particular significance was the rapid development and retention of a layer of flocculated biomass above the expanded–bed of GAC in the experimental reactor. S.E.M. observations indicated the formation of biomass granules up to 0.3 mm in size. Growth of the biomass layer and absence of a complete biofilm confirmed that the GAC was transferring biological activity into the supernatant. Methanogenic

activity within the GAC bed was vital in preventing wash–out of the flocculated/granular biomass. Re–seeding of the supernatant with active culture and hydraulic stabilization of a bed of flocculated biomass are two important mechanisms by which GAC was observed to enhance the treatment of phenolic wastewaters. Both have significant implications on the design of industrial wastewater treatment processes.

ACKNOWLEDGEMENTS

I would like to thank my graduate supervisor, Dr. Murray Gray as well as Dr. Phil Fedorak and Dr. Steve Hrudey, who acted as co–supervisors, for the help, advice and encouragement they provided during the course of this project. I would also like to express thanks to the staff and graduate students of the Department of Microbiology at the University of Alberta for their cooperation and assistance which allowed me to carry out my experimental work. Neil Fairbairn and Debbie Roberts, in particular, were extremely helpful to me in my endeavors with anaerobic bacteria. I would finally like to thank all the other staff members at the University of Alberta who provided me with assistance with my work.

Financial support for this project was provided by a strategic grant from the Natural Sciences and Engineering Council of Canada. NSERC also provided the bulk of my personal funding, in the form of a graduate scholarship, during my stay at the University of Alberta .

TABLE OF CONTENTS

Chapter			Page
	ABSTRACT		iv
	AKNOWLEDGEMENTS		vi
	TABLE OF CONTENTS		vii
	LIST OF TABLES		xi
	LIST OF FIGURES		xiii
	NOMENCLATURE		xvii
1.	INTRODUCTION		1
	1.1	Background	1
	1.2	Project Objectives	3
	1.3	Outline of Experimental Program	4
	1.4	Thesis Structure	6
2.	OVERVIEW OF THE ANAEROBIC DIGESTION PROCESS		7
	2.1	Advantages and Disadvantages	7
	2.2	Microbiology	10
	2.3	Advanced Treatment Reactor Designs	14
3.	LITERATURE REVIEW		20
	3.1	Degradation of Phenol	20
	3.2	Role of GAC in Treatment of Phenolic Wastewaters	26
	3.3	Granulation in Anaerobic Treatment Reactors	38
4.	EXPERIMENTAL METHODS		44
	4.1	Anaerobic Culture Techniques	44
	4.2	Culture Medium	44
	4.3	Serum Bottle Procedures	47
	4.4	Phenol Analysis	48

4.5 Acetic Acid Analysis 49

4.6 Methane Analysis 49

4.7 Scanning Electron Microscopy 50

4.8 Volatile Suspended Solids 51

4.9 Thermogravimetric Analysis 52

5. BIOREACTOR STUDY 53

5.1 Experimental Bioreactor Design 53

5.2 Pseudo Steady–State Kinetic Analysis 56

5.3 Procedures 61

5.3.1 Bioreactor Start–Up 61

5.3.2 Trial Period 63

5.3.3 Continuous Operation 66

5.3.4 Degradation Kinetics of Bioreactor Inoculum 69

5.4 Results 70

5.4.1 CFRB Runs 70

5.4.2 Degradation Kinetics of Bioreactor Inoculum 85

5.4.3 Mass Balance on Phenol 90

5.5 Discussion 93

6. SHOCK LOADING EXPERIMENTS 99

6.1 Review of Objective 99

6.2 Procedures 101

6.2.1 Bioreactor Preparation 101

6.2.2 Flocculated Biomass Culture Set–Up 101

6.2.3 GAC Culture Set–Up 102

6.2.4 Phenol Adsorption Isotherm 104

6.3 Results and Discussion 105

6.3.1 Shock Loading of Flocculated Biomass Cultures 105

6.3.2 Adsorption Isotherms 118

6.3.3 Shock Loading of GAC Cultures 121

6.3.4 Comparison of GAC and FLOC Culture Responses 130

7. ADDITIONAL STUDIES ON THE GAC AND FLOC CULTURES 134

7.1 Scanning Electron Microscopy 134

7.1.1 GAC Particles from Bioreactor 134

7.1.2 Flocculated Biomass from Bioreactor 139

7.1.3 Summary and Discussion 143

7.2 Thermogravimetric Analysis 147

7.2.1 Biomass Measurement 147

7.2.2 Procedures 149

7.2.3 Sample Preparation 150

7.2.4 Results 151

7.2.5 Error Analysis 157

7.2.6 Discussion 159

7.3 Phenol Adsorption Kinetics 162

7.3.1 Objective 162

7.3.2 Procedures 163

7.3.3 Results and Discussion 164

7.4 FLOC Bed Reactor 167

7.4.1 Objective 167

7.4.2 Procedures 168

7.4.3 Results and Discussion 169

8. SUMMARY AND DISCUSSION 171

8.1 Achievement of Objectives 171

8.2 Implications on Treatment Reactor Design 174
9 CONCLUSIONS 176
10 RECOMMENDATIONS FOR FUTURE WORK 178
REFERENCES 180
APPENDICES
I Bioreactors: Equipment 185
II Data and Calculations for Bioreactor Study 202
II.A Pseudo Steady–State CFRB Data 202
II.B CFRB Data: Batch Phase 205
II.C Calculations For CFRB Kinetic Analysis 207
II.D Data and Calculations For 2–L Digester 210
II.E Analysis of CSTR Dynamics 212
II.F Pseudo Steady–State Error Analysis 214
II.G Phenol Balance Calculations 218
II.H Additional Data For Phenol Balance 227
III Shock Loading Experiment: Additional Figures 228

LIST OF TABLES

Table	Description	Page
TABLE 3.1	Concentration of Various Phenolics and VOA's in H–Coal Liquefaction Wastewater	21
TABLE 3.2	Studies Employing Granular Activated Carbon	28
TABLE 4.1	Composition of Stock Solution Used in Culture Medium	45
TABLE 4.2	Composition of Complete Culture Medium	46
TABLE 5.1	Pseudo Steady–State Data From Continuous Phase of CFRB Runs	72
TABLE 5.2	Breakdown of Phenol–Degrading Activities in Reactor During CFRB Runs	81
TABLE 5.3	Biomass Content of Reactor at End of CFRB Runs	83
TABLE 5.4	Specific Phenol–Degrading Activities in CFRB Runs	86
TABLE 5.5	Summary of Phenol Balance on CFRB Runs	91
TABLE 6.1	Pre–Shock Load Phenol–Degrading Activities in FLOC Cultures	111
TABLE 6.2	Post–Shock Load Phenol–Degrading Activities in FLOC Cultures	113
TABLE 6.3	Specific Phenol–Degrading Activities in Shock Loaded FLOC Cultures	114
TABLE 6.4	Specific Phenol–Degrading Activities in Shock Loaded GAC Cultures	128
TABLE 7.1	Results of Thermogravimetric Analysis of Sample GAC and Control GAC	152
TABLE 7.2	Thermogravimetric Analysis of Anaerobic Biomass Standard	155
TABLE 7.3	Elemental Analysis of GAC and Anaerobic Biomass	161
TABLE II.A.1	CFRB Data: Run 1	202
TABLE II.A.2	CFRB Data: Run 2	203

TABLE II.A.3	VSS Concentration in Effluent Collected	204
TABLE II.B.1	Batch Degradation Data: CFRB Run 1	205
TABLE II.B.2	Batch Degradation Data: CFRB Run 2	206
TABLE II.D.1	Data From Batch Degradation in 2–L Digester	210
TABLE II.F.1	Phenol Concentration Data: Last 3 Days of CFRB Run 1	214
TABLE II.F.2	Phenol Concentration Data: Last 3 Days of CFRB Run 2	216
TABLE II.G.1	Elemental Composition of Biomass	219
TABLE II.H.1	Data from CFRB Run 0	227
TABLE II.H.2	VSS Concentration in Effluent: CFRB Run 0	227

LIST OF FIGURES

Figure	Description	Page
FIGURE 2.1	Major Stages of Anaerobic Methanogenic Degradation	11
FIGURE 2.2(a)	Attached Growth Reactor Designs	17
FIGURE 2.2(b)	Agglomerated Growth Reactor Designs	17
FIGURE 3.1	Proposed Pathway for the Anaerobic Degradation of Phenol	24
FIGURE 3.2(a)	Phenol Biodegradation in Sequential–Batch Cultures After 1st Supernatant Withdrawal	35
FIGURE 3.2(b)	Phenol Biodegradation in Sequential–Batch Cultures After 2nd Supernatant Withdrawal	36
FIGURE 3.3	Phenol Adsorption on GAC (Without Bacteria) and GAC From Serum–Bottle Cultures (In Presence of Attached Growth)	37
FIGURE 5.1	Schematic of Experimental Reactor	54
FIGURE 5.2	Locations of Flocculated Biomass in Reactor Column	64
FIGURE 5.3	Pseudo Steady–State Phenol and Acetic Acid Concentration Profiles With Time in CFRB RUN 1	73
FIGURE 5.4	Pseudo Steady–State Phenol and Acetic Acid Concentration Profiles With Time in CFRB RUN 2	74
FIGURE 5.5	Measured Cumulative Total Dry Gas Production During Pseudo Steady–State Phase of CFRB Run 1	76
FIGURE 5.6	Measured Cumulative Gas Total Dry Gas Production During Pseudo Steady–State Phase of CFRB Run 2	77
FIGURE 5.7	Phenol Degradation in Bioreactor Tank During Batch Phase of CFRB Run 1	79
FIGURE 5.8	Phenol Degradation in Bioreactor Tank During Batch Phase of CfRB Run 2	80

FIGURE 5.9	Batch Phenol Degradation in Bioreactor Inoculum Source	88
FIGURE 5.10	Measured Cumulative Total Dry Gas Production in 2–L Digester	89
FIGURE 5.11	Phenol Concentration in Bioreactor During Pseudo Steady–State Phase of CFRB Run 1	96
FIGURE 5.12	Phenol Concentration in Bioreactor During Pseudo Steady–State Phase of CFRB Run 2	97
FIGURE 6.1	Response of FLOC Culture 1 to a Shock Load of 980 mg/L	106
FIGURE 6.2	Response of FLOC Culture 2 to a Shock Load of 1430 mg/L	107
FIGURE 6.3	Response of FLOC Culture 3 to a Shock Load of 1850 mg/L	108
FIGURE 6.4	Pre–Shock Load Batch Activity in FLOC Culture 1	110
FIGURE 6.5	Post–Shock Load Batch Activity in FLOC Culture 1	112
FIGURE 6.6	Measured Cumulative Net Dry Gas Production in Shock Loaded FLOC Cultures	117
FIGURE 6.7	Data for Phenol Adsorption Isotherms of GAC From Bioreactor and Clean GAC	119
FIGURE 6.8	Response of GAC Culture 1 to a Shock Load of 1130 mg/L in the Supernatant	123
FIGURE 6.9	Response of GAC Culture 2 to a Shock Load of 1540 mg/L in the Supernatant	124
FIGURE 6.10	Response of GAC Culture 3 to a Shock Load of 2170 mg/L in the Supernatant	125
FIGURE 6.11	Pre–Shock Load Activity: GAC Culture 1	126
FIGURE 6.12	Post–Shock Load Activity: GAC Culture 1	127
FIGURE 6.13	Measured Cumulative Net Dry Gas Production in Shock Loaded GAC Cultures	131
FIGURE 7.1(a)	Scanning Electron Micrograph of Colonized GAC From Bioreactor	135
FIGURE 7.1(b)	Scanning Electron Micrograph of Colonized GAC From Bioreactor	135

FIGURE 7.2(a)	Scanning Electron Micrograph of Colonized GAC From Bioreactor	136
FIGURE 7.3(a)	Scanning Electron Micrograph of Colonized GAC From Bioreactor	137
FIGURE 7.3(b)	Scanning Electron Micrograph of Colonized GAC From Bioreactor	137
FIGURE 7.4(a)	Scanning Electron Micrograph of Granular Biomass From Bioreactor	140
FIGURE 7.4(b)	Scanning Electron Micrograph of Granular Biomass From Bioreactor	140
FIGURE 7.5(a)	Scanning Electron Micrograph of Granular Biomass From Bioreactor	141
FIGURE 7.5(b)	Scanning Electron Micrograph of Granular Biomass From Bioreactor	141
FIGURE 7.6(a)	Scanning Electron Micrograph of Granular Biomass From Bioreactor	142
FIGURE 7.6(b)	Scanning Electron Micrograph of Granular Biomass From Bioreactor	142
FIGURE 7.7	Reproduction of Chart Recorder Output From Typical TGA Determination of Sample GAC	154
FIGURE 7.8	Kinetics of Phenol Adsorption onto GAC in Bioreactor Determined *in Situ*	165
FIGURE I.1	Reactor Tank and Fittings	187
FIGURE I.2	Reactor Column	188
FIGURE I.3	Recirculation Pump and Fittings	191
FIGURE I.4	Feed System	192
FIGURE I.5	Gas Collection System	194
FIGURE I.6	Effluent Collection System	196
FIGURE I.7	Liquid Sampling System	199
FIGURE III.1	Pre–Shock Load Batch Activity: FLOC Culture 1	229
FIGURE III.2	Post–Shock Load Batch Activity: FLOC Culture 2	230

FIGURE III.3	Pre–Shock Load Batch Activity: FLOC Culture 3	231
FIGURE III.4	Pre–Shock Load Activity: GAC Culture 2	232
FIGURE III.5	Post–Shock Load Activity: GAC Culture 2	233
FIGURE III.6	Pre–Shock Load Activity: GAC Culture 3	234
FIGURE III.7	Post–Shock Load Activity: GAC Culture 3	235

NOMENCLATURE

auth.	=	authentic
BS	=	Berl saddles
C	=	effluent substrate concentration in chemostat
C_e	=	adsorption equilibrium concentration of solute
C_a	=	concentration of solute in liquid addition in adsorption isotherm assay.
C_o	=	feed substrate concentration in chemostat
CCWW	=	coal conversion wastewater
CF	=	correction factor for thermogravimetric analysis
CFRB	=	continuous–flow recylce bioreactor
COD	=	chemical oxygen demand
CONC.	=	concentration
D	=	dilution rate
dd	=	double distilled
df	=	degrees of freedom
dil.	=	dilution
δx	=	absolute uncertainty on estimate of quantity x
EBR	=	expanded–bed reactor
F	=	volumetric flowrate in chemostat reactor
FBR	=	fluidized–bed reactor
FLOC	=	flocccculated biomass
GAC	=	granular activated carbon
GC	=	gas chromatography
HAc	=	acetic acid
HP	=	Hewlett–Packard

HPLC	=	high performance liquid chromatography
k	=	pre–exponential constant in Freundlich iostherm equation
K_i	=	substrate inhibition constant
K_s	=	Monod kinetic half–rate constant
m	=	sample mass in thermogravimetric analysis
m_b	=	mass of total biomass in chemostat
m_c	=	mass of activated carbon
m_f	=	mass of flocculated biomass in chemostat
m_p	=	total mass of phenol in serum bottle cultures
$\Delta \overline{m}$	=	percent change in sample mass in thermogravimetric analysis
Δ m	=	absolute change in sample mass in thermogravimetric analysis
μ	=	specific growth rate of bacteria
n	=	order of sustrate inhibition
1/n	=	exponential constant in Freundlich isotherm equation
n_1, n_2	=	number of samples of random variables x_1 and x_2
NAC	=	non–activated carbon
PBR	=	packed(fixed)– bed reactor
q	=	mass adsorbate per mass adsorbent at equilibrium
r	=	correlation coefficient of linear regression analysis
R	=	reaction rate
rpm	=	revolutions per minute
RR	=	Raschig rings
s	=	standard deviation
S	=	substrate concentration
S.E.M.	=	scanning electron microscopy

synth.	=	synthetic
S_p	=	calculated parameter for confidence interval determination
t	=	time
$t_{\alpha/2}$	=	Students t–distribution parameter
T_o	=	initial temperature in thermogravimetric analysis
T_1	=	drying temperature in thermogravimetric analysis
T_2	=	volatilization temperature in thermogravimetric analysis
TGA	=	thermogravimetric analysis
UASB	=	upflow anaerobic sludge blanket
V	=	substrate biodegradation activity (rate)
V_a	=	volume of concentrated phenol solution added in adsorption isotherm assay
V_c	=	biodegrading activity of GAC supported culture
$\bar{V}_c$	=	specific biodegrading activity of GAC immobilized culture
V_f	=	biodegrading activity of flocculated culture
$\bar{V}_f$	=	specific biodegrading activity of flocculated culture
V_l	=	liquid volume hold–up in chemostat
V_l	=	supernatant liquid volume in serum–bottles
V_{max}	=	maximum substrate biodegrading activity
V_o	=	overall biodegrading activity of chemostat
$\bar{V}_o$	=	specific overall biodegrading activity of chemostat
V_s	=	biodegrading activity of suspended culture

∇_s	=	specific biodegrading activity of suspended culture
VOA	=	volatile organic acid
VS	=	volatile solids
VSS	=	volatile suspended solids
X	=	suspended biomass concentration in a chemostat
$x_{b/c}$	=	biomass concentration on GAC (mass VS/mass dry GAC)
$X_{b/c}$	=	biomass concentration on GAC [mass VS/(mass VS + mass dry GAC)]
$\bar{x}_1, \bar{x}_2$	=	means of random variables x_1 and x_2
$Y_{x/s}$	=	biomass yield (mass biomass/mass substrate)
$1-\alpha$	=	confidence interval

1 INTRODUCTION

1.1 BACKGROUND

Application of the anaerobic digestion process to the treatment of industrial wastewaters has attracted significant attention in recent years. Anaerobic digestion is a biological process in which organic compounds are reduced, mainly to methane and carbon dioxide gases, by a complex group of bacteria in an environment devoid of oxygen. The anaerobic process has been used since the last century for the reduction of domestic sewage sludge and has also been successfully adopted for the treatment of wastewaters arising from the food and beverage industries. Effort in developing the process for production of biogas via the anaerobic degradation of agricultural waste and municipal refuse was inspired by the energy crisis of the early 1970's and the subsequent search for cheap fuel alternatives. It is only in the last decade or so that the anaerobic process has been considered for the treatment of aqueous effluents from chemical, petrochemical, petroleum and coal industries.

Phenol and phenolic compounds are prevalent in the effluents of the following industrial operations:

1. coal conversion, *i.e.* liquefaction and gasification,
2. petrochemical processes, especially those involving phenolics as raw materials, intermediates or end products, *i.e.* phenolic resin manufacturing,
3. petroleum refining,

as well as others. The Environmental Protection Agency in the U.S. lists phenol and several phenolic derivatives, including isomers of cresol, dimethylphenol, chlorophenol and nitrophenol, as priority pollutants. As well as increasing the biochemical oxygen demand of receiving waters, even very small concentrations (ppm) of phenolics are toxic to aquatic life and can cause taste and odor problems in drinking water.

Appropriate methodologies for treatment of industrial, phenolic wastewaters vary widely, being a function of wastewater composition, strength, flowrate and required effluent quality. Options for treatment include physical and chemical methods as well as the aerobic and anaerobic biological processes. Complete removal of phenolics from wastewater using physical or chemical methods such as solvent extraction, oxidation, air stripping and adsorption is often uneconomical. Biological processes represent attractive alternatives from an economic standpoint. Phenolic wastewaters, like most industrial wastewaters, often contain a complex mixture of organic and inorganic compounds, many of which inhibit or are toxic to bacteria above certain concentration levels, and are difficult to treat biologically. In fact, phenolic compounds themselves have often been considered to be difficult to degrade. Combined treatment strategies are often most effective for the treatment of high strength wastewaters. Toxic and inhibitory compounds are reduced to levels acceptable for biological treatment by a physical or chemical method and are subsequently degraded to even lower levels in a biological step. Final polishing of the effluent in a physical or chemical step, such as activated carbon adsorption or ozone oxidation, may be required to meet effluent standards. Traditionally, the aerobic biological process, specifically the extended aeration activated sludge process, has been employed for treatment of phenolic wastewaters.

In the biological treatment of industrial wastewaters, including phenolic wastewaters, the anaerobic process offers certain potential advantages over its more established aerobic counterpart. Most importantly, excess sludge production is lower and energy efficiency is greater. Difficulties in application have arisen primarily as a result of a poor understanding of the complex microbial interactions involved and a lack of a rational basis for optimizing the design and operation of treatment reactors. Research has resulted in significant improvements in these areas and has fostered a renewed interest in the anaerobic process. Advanced

reactor design concepts have been developed with the emphasis on increasing bacterial retention times to offset the characteristic slow growth of anaerobes. The slow growth rate is often considered to be the major drawback of the process, resulting in protracted reactor start–up times and process instabilities. Immobilization of bacteria by attachment to an inert support is a common method of increasing retention times in biological reactors. Agglomeration of dispersed bacteria into flocs and the formation of granules of biomass with good settling properties is another method.

1.2 PROJECT OBJECTIVES

Granular activated carbon (GAC) has proven to be an effective bacterial support in attached–growth treatment reactor systems. The inherent adsorption capacity of activated carbon will protect a biological system against the harmful concentrations of toxic and/or inhibitory compounds in the wastewater feed which may be present during normal operation or as a result of process upsets. *In situ* use of GAC can, therefore, improve the stability of the process. The degree to which the use of GAC contributes to the improvement of the anaerobic bio–treatment of phenolics has not been fully appreciated. It has been proposed that GAC plays an additional role in process enhancement aside from functioning as a simple support medium and adsorbent buffer. Immobilization of bacteria has often been suggested as a reason for the stimulation of substrate degradation kinetics. It is possible that this effect is present in anaerobic systems incorporating GAC as a support medium.

The present study attempted to elucidate further the mechanisms by which GAC serves to enhance the anaerobic degradation of phenolics apart from its role as a physical adsorbent. Specifically, the project objectives were:

1. to establish the effect of immobilization of an anaerobic, phenol–degrading enrichment culture on GAC cn the kinetics of phenol degradation,

2. to establish the characteristics of the immobilized culture which are important in the design of treatment reactors,
3. to determine the relationship between the use of GAC as a support medium and the development and retention of unsupported biomass in treatment reactors.

In order to achieve these objectives, an experimental program, involving the operation of laboratory–scale anaerobic bioreactors, was undertaken. GAC was incorporated into the reactor design as a bacterial support.

1.3 OUTLINE OF EXPERIMENTAL PROGRAM

A steady–state experiment was designed to overcome the difficulties inherent in using batch reactors to determine bio–degradation kinetics of systems incorporating GAC as a microbial support. A continuous–flow recycle bioreactor (CFRB) was employed in which a phenol–degrading anaerobic culture was immobilized by attachment to GAC. The GAC particles were maintained in the reactor as an expanded bed. To eliminate wastewater composition as an uncontrolled variable, a well–defined model wastewater containing a synthetically prepared mixture of inorganic nutrients and phenol, as the major carbon source, served as the reactor feed. Phenol concentrations experienced by the culture within the reactor were maintained below inhibitory levels.

Originally, the experimental objectives were restricted to a study of the properties of the attached culture. Shortly after start–up of the experimental bioreactor, however, a significant quantity of biomass was observed to be accumulating in the reactor as flocculated and settled material. Visual observations suggested the onset of granulation. Retention of this active, flocculated biomass could have implications on the design of treatment reactors. It was, therefore, decided that the phenomenon merited further investigation. The objectives were

expanded to include a study of the properties of the flocculated biomass and the factors involved in its retention.

The resulting research program consisted of three related experimental studies which are briefly described here.

1. Bioreactor Studies

The specific phenol degradation kinetics of the attached and flocculated cultures were determined in the experimental bioreactor. The objective was to compare the kinetics of the attached, flocculated and suspended bacteria and to determine if attachment to GAC or formation of flocs enhanced the specific phenol degrading capability of the bacteria.

2. Shock Loading Experiments

GAC–attached and flocculated biomass from the bioreactor were subject to spikes of inhibitory phenol concentration in batch culture experiments in order to determine the relative abilities of the two culture types to respond to these stresses.

3. Additional Studies of the GAC–Attached and Flocculated Cultures

Qualitative information on the nature and extent of the attached growth on GAC removed from the bioreactor was provided by scanning electron microscopy. A newly developed technique based on thermogravimetric analysis enabled a direct measurement of the quantity of attached biomass. *In situ* measurements of phenol adsorption kinetics were carried out with the purpose of providing an indirect assessment of the effect of different hydraulic regimes within the reactor on the attached culture.

Scanning electron microscopy was used to characterize qualitatively the biomass flocs which developed in the bioreactor and to determine if granulation had

occurred. A reactor containing only flocculated biomass and no GAC was operated with the objective of elucidating the mechanisms that accounted for the excellent retention of the flocculated biomass that was experienced in the GAC reactor. Of specific interest was the role of GAC in helping to establish and/or retain this biomass.

1.4 THESIS STRUCTURE

This thesis report is comprised of two main parts. The first part provides background material relevant to the project. Chapter 2 is a brief overview of the anaerobic microbial process and the current technology in anaerobic treatment of wastewaters. Chapter 3 is a review of the literature dealing with the anaerobic treatment of phenolic wastewaters with an emphasis on the role of GAC as a biological support. Literature on microbial granulation in anaerobic systems is also reviewed. The second part of the report describes the experimental program in detail, including procedures, presentation and analysis of results, and discussion. A separate chapter has been dedicated to each of the three experimental studies described previously (Chapters 5, 6 and 7). Key results and observations are combined and summarized and the implications on process technology are discussed in Chapter 8. Conclusions and recommendations for future work are then presented in Chapters 9 and 10, respectively.

2 OVERVIEW OF THE ANAEROBIC DIGESTION PROCESS

The objective of this chapter is to provide a brief overview of the anaerobic digestion process in industrial wastewater treatment. The discussion will be limited to the methanogenic anaerobic process which is responsible for the degradation of organic matter to methane and carbon dioxide gases although non–methanogenic processes, such as microbial sulfate reduction, are also important in anaerobic waste treatment (Stronach *et al.*, 1986). The anaerobic conversion of organic substrates is carried out in a sequence of tightly coupled hydrolysis, oxidation and reduction reactions that are catalyzed by a complex consortium of closely interacting bacteria. In nature, methanogenic processes play an important role in the carbon cycle, contributing to the degradation of dead organic matter. Methanogenic bacteria are responsible for the decay of vegetation in swamps and bogs and the production of swamp gas containing methane. Methane production in the rumen of cows also occurs via the methanogenic process. Engineered application of methanogenic processes has been almost entirely restricted to the degradation of both solid and aqueous wastes resulting from domestic, agricultural and industrial operations and the associated production of biogas. The following sections will cover the advantages and limitations of the anaerobic process in industrial wastewater treatment, the basic microbial interactions involved and concepts in advanced treatment reactor design.

2.1 ADVANTAGES AND DISADVANTAGES

The benefits of the anaerobic process must be assessed in comparison to aerobic biological treatment. Although both processes utilize the metabolic abilities of microorganisms, primarily bacteria, to degrade undesired organic constituents, fundamental differences between the two exist. The biochemical degradation

pathways and the nature of the bacteria involved are remarkably different. The anaerobic processes are characterized by reductive pathways and oxidative pathways that employ terminal electron acceptors other than molecular oxygen. The anaerobic consortium responsible for the degradation of a particular organic compound is far more complex than the single aerobic organism which is often capable of degrading the same organic. In general, growth rates and substrate utilization rates of the anaerobic consortia are much lower than those of aerobic bacteria (Sahm, 1984). This is a result of the low efficiency of energy utilization in anaerobic metabolic pathways. For instance, anaerobic bacteria are only able make use of 5% – 7% of the chemical energy of organic substrates in the production of new cell material (Sahm 1984). Most of the remaining chemical energy is converted into end products (*i.e.* methane and carbon dioxide).

The principle advantages of the anaerobic process which make it an attractive alternative to aerobic treatment are:

1. lower biomass production
2. lower nutrient requirements
3. potential for greater energy efficiency.

According to Sahm (1984) aerobic bacteria typically convert 50% of the organic substrate in the wastewater into more cells or biomass. The production of this biomass, termed excess sludge, usually represents a significant solids waste disposal problem in itself. In anaerobic processes, on the other hand, only about 5% of the substrate is converted to biomass while the remainder is catabolized to product gas. In addition, anaerobic sludge has better characteristics for waste disposal (Obayashi and Gordan, 1985).

The lower nutrient requirement per quantity of substrate degraded is also a result of the lower cell yield. In addition to a carbon source, nutrients such as nitrogen, phosphorus, sulphur, certain vitamins and trace amounts of metal ions are

generally required for growth of bacteria. Most industrial wastewaters require nutrient supplementation and this can be a considerable expense to the treatment process. The amount of supplementation is proportional to the cell yield of the bacteria.

Industrial–scale aerobic–treatment reactors require aeration for efficient operation. Providing large quantities of air to ensure adequate aeration and mixing represents a significant operating cost in aerobic–treatment processes. Anaerobic processes have no such requirement. In addition, the product of anaerobic degradation is a gas typically containing between 50% and 90% methane. The energy content of this gas can be recovered and used to provide the energy requirements, *i.e.* heating, mixing and pumping, for the treatment system. Treatment processes employing anaerobic digestion have the potential for energy self–sufficiency (*i.e* Van den Berg, 1984).

The principle limitations of the anaerobic process are:

1. start–up and recovery times are long,
2. the microbial process is complex and poorly understood,
3. it is a pretreatment process because unconverted organics remain in the wastewater.

The low energy efficiency of anaerobic metabolism means that the growth rates are low. Minimum doubling times of the anaerobic bacteria in treatment processes are typically on the order of days (Henze and Harremoes, 1983) whereas those of aerobes are generally much lower, on the order of minutes or hours. This low growth rate means that the time required to develop an active culture in a large treatment reactor from a small inoculum is long. For the same reason, the recovery from process upsets which result in a loss of viable biomass is slow.

The microbiology of anaerobic degradation and the nature of the interactions between the individual bacterial species involved have not been fully elucidated. As

a consequence, the causes of process instabilities and failures are frequently misunderstood. This makes it difficult to establish a rational basis for monitoring and controlling the process.

As a result of these limitations, research emphasis has been on improving the understanding of the microbiology of anaerobes and elucidating the specific degradation pathways, as well as on the development of reactor designs which increase bacterial retention. In the treatment of industrial wastewaters, the anaerobic process has been recognized as essentially a pretreatment process. Although it has been demonstrated that bulk organic removal at high rates is possible, further treatment of effluent to remove nitrogen compounds, such as ammonia and nitrate, or residual organics is usually required. A final aerobic or chemical/physical polishing step can achieve this.

2.2 MICROBIOLOGY

The stages in the anaerobic degradation of organic matter have been described by numerous reviewers (*i.e.* Sahm, 1984, Stronach *et al.*, 1986, Wilkie and Colleran, 1988). Sahm (1984) describes three major groups of bacteria performing three major steps in the overall degradation of complex organics to methane and carbon dioxide as in Figure 2.1.

STAGE 1: Hydrolysis and Acetogenesis

Large organic polymers *i.e.* proteins, polysaccharides, fats, etc. are first broken down into smaller subunits and solubilized by hydrolytic enzymes secreted by the bacteria. Soluble organics are fermented and anaerobically oxidized to acetate, hydrogen, carbon dioxide or higher organic acids and alcohols. A complex group of both facultative and strictly anaerobic bacteria are involved in this stage of the process, the composition of which is dependent on the type of substrate or substrates in the waste. Very little is known about the individual species involved

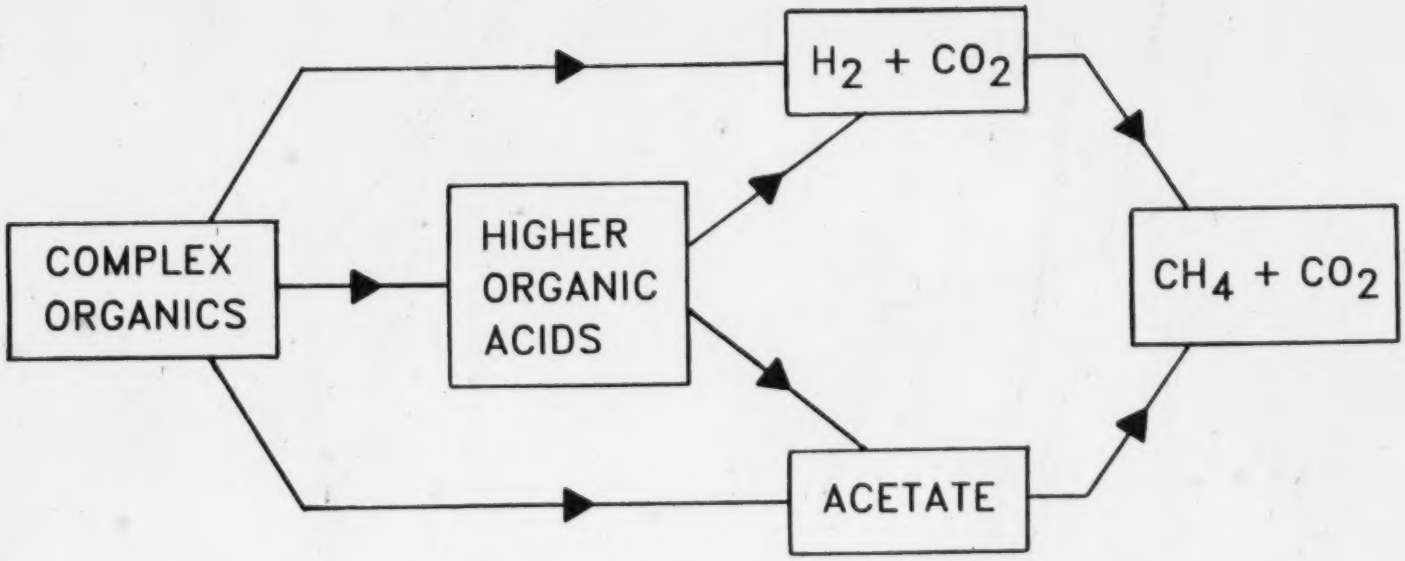


FIGURE 2.1: Major Stages in Methanogenic Anaerobic Degradation

and in most cases they have not been isolated or characterized. They are often referred to as the hydrolytic, fermentative bacteria and acetogenic bacteria.

STAGE 2: Acetogenesis

The higher organic acids *i.e.* propionate, butyrate, and longer chain acids, and alcohols, *i.e.* ethanol, are oxidized to acetate, carbon dioxide and hydrogen by a group of strictly anaerobic bacteria called the obligate hydrogen–producing acetogens. This is a newly discovered group of bacteria, a few species of which have been recently identified. The hydrogen producing reactions catalyzed by these bacteria are thermodynamically unfavorable unless the concentration of molecular hydrogen is kept extremely low. For instance, the degradation of propionate to hydrogen, carbon dioxide and acetate will only proceed if the partial pressure of the hydrogen product in the digester is maintained between 10^{-4} and 10^{-6} atm (McCarty and Smith, 1986). The metabolic activities of these bacteria must therefore be coupled to the consumption of hydrogen by the methanogenic bacteria. Efficient inter–species transfer of hydrogen between the two different bacteria is crucial to the success of the degradation process.

STAGE 3. Methanogenesis

The final stage of the degradation process is the production of methane and carbon dioxide by the methanogens. Many species of methanogens have now been isolated from wastewater treatment reactors and natural environments and characterized. Taylor (1982) lists 17 species as being isolated in pure culture and others have been identified since. They are members of a unique family of bacteria, the archaebacteria, which are quite distinct from typical procaryotes, and are very strict anaerobes, requiring redox potentials below – 330 mV for growth and metabolism (Hungate, 1969). Maintenance of such an oxygen–free environment in a treatment reactor depends on the oxygen scavenging action of facultative anaerobes present in the consortium. The range of substrates which the methanogens are able

to utilize is quite limited. The two major classes of these bacteria are the acetoclastic, or acetate–utilizing, and hydrogenotrophic, or hydrogen–utilizing, methanogens. About 70% of the methane produced in anaerobic processes is from the breakdown of acetate. Other substrates reported to have been utilized by methanogens are formate and methanol. Higher organic acids and alcohols are not degraded directly by the methanogens but must first be broken down by the acetogenic bacteria.

Continued successful degradation of organic substrates to gaseous products requires a careful balance of active populations of the bacteria performing the various reactions. The failure or partial failure of any single species to perform its individual task results in the accumulation of inhibitory intermediates. The organic acids are considered to be strong inhibitors of methanogenesis and rising concentration of these compounds in treatment reactors is an indication of potential process failure. The tight coupling of the metabolic activities of the acetogenic and methanogenic bacteria illustrates the degree of symbiosis displayed by the anaerobic consortium. The acetogens provide the necessary substrates for the methanogens and in turn depend on the activity of methanogens to maintain an environment of low hydrogen concentration. The degradation of organic acids to methane and carbon dioxide is generally considered to be the rate–limiting step in the degradation of soluble organics. This is a reflection of the low metabolic rates of the acetogenic and methanogenic species.

As with all biological processes, the anaerobic process is sensitive to environmental conditions. Important environmental conditions in wastewater treatment processes are:

1. temperature,
2. pH,
3. wastewater composition.

Most of the bacteria in anaerobic treatment are mesophilic, preferring an intermediate temperature range (*i.e.* 25°– 45°C). Lin *et al.* (1987) reported an optimum temperature of 35°C for the methanogenic degradation of volatile organic acids. Adjustment of wastewater temperature may be required for efficient operation. The pH for optimum growth of the methanogenic consortium is in the neutral range, between 6 and 8 (Sahm, 1984). A healthy culture creates a buffering capacity by the production of bicarbonate which enables the system to counteract a reduction in pH caused by a build–up of organic acids produced by the acetogenic bacteria. This buffering capacity is, however, limited. For many industrial wastewaters pH adjustment may be required.

The treatability of an industrial wastewater by biological means is a strong function of the wastewater composition. As was mentioned earlier, the wastewater may lack an appropriate balance of nutrients. As well as the macronutrients required for growth and energy of the anaerobic bacteria, such as carbon, nitrogen, phosphorus and sulphur, the requirement of certain micronutrients has been identified. Examples are the trace metals such as nickel, molybdenum, selenium and tungsten as well as iron (Stronach *et al.*, 1986). Industrial wastewaters often contain compounds which are toxic or inhibitory to anaerobic bacteria above certain concentration levels. These include sulphide, cyanide, thiocyanate, ammonia and heavy metals. Most aromatic compounds, including phenolics, are inhibitory substrates. Given an appropriate acclimation time and procedure, many such compounds are eventually degraded by anaerobic cultures.

2.3 ADVANCED TREATMENT REACTOR DESIGN

The greatest need in anaerobic treatment of industrial wastewaters is for reactor designs that:

1. reduce start–up times,

2. increase process stability.

The slow growth rate and the time required to acclimate a starter culture to a particular industrial wastewater combine to increase the length of time for reactor start–up. Process stability requires that a high concentration of active, acclimated bacteria be maintained in the reactor during process upsets. Wastewater treatment systems routinely experience wide fluctuations in both hydraulic and organic loadings. In particular, these systems are prone to shock loadings. To be effective, a treatment system must be able respond to and recover quickly from these upsets.

In response to these problems, the strategy employed in advanced treatment reactor design is to increase the retention time of the bacteria. The importance of bacterial retention is illustrated by considering the simplest of reactor designs, the well–mixed digester. This is a continuous, stirred–tank reactor in which the bacteria are uniformly dispersed in the liquid medium. Suspended bacteria are continuously washed out with the reactor effluent. The mass balance equation for biomass is:

$$\frac{dX}{dt} = (\mu - D) \cdot X \qquad (2.1)$$

where μ is the specific growth rate of the bacteria, D is the dilution rate (inverse of hydraulic residence time) and X is the reactor biomass concentration (Bailey and Ollis, 1986). Hence, the only non–trivial steady–state has a dilution rate fixed by the growth rate. Low growth rates result in large residence times and, for a fixed volumetric flowrate of wastewater, large reactor volumes. The constant wash–out of biomass means that it is also an inherently unstable design. A process upset, such as a nutrient imbalance, a high concentration of inhibitory compounds or a temperature shock, that causes a transient reduction in the growth rate will result

in a decline of the bacterial content of the reactor if the volumetric flowrate remains fixed. If this loss of active culture persists the entire population will eventually be washed out of the reactor.

Decoupling of the dilution rate from the growth rate is achieved by cell immobilization. This technique is commonly used in bioreactor design to increase the retention time of cells by physically separating them from the liquid. Two methods are employed in establishing this separation in anaerobic treatment reactors. The first is the agglomeration of biomass into particles with good settling characteristics, often in combination with recycle of biomass. The second method is by attachment of the biomass to an support medium. Fortunately, the anaerobic bacteria have a strong tendency to adhere both to themselves, forming flocs and granules, and to solid surfaces. In doing so, the bacteria secrete a polymer material which may aid in the adhesion process by acting as a binding agent (Stronach *et al.*, 1986). The secretion of polymer in attached growth biological systems is common and the exo–polymer is referred to as a glycocalyx (Costerton *et al.*, 1978). Coverage of a surface by a uniform layer of bacterial growth is a process known as biofilm formation. Flocculation and biofilm formation occur in many cell systems and are particularly useful in anaerobic reactor designs.

Three basic attached growth reactor designs are shown in Figure 2.2(a). Packed–bed reactors are often called anaerobic filters because the bacteria tend to become trapped in the interstices of the packing. The anaerobic filter process was first developed by Young and McCarty (1969). A variety of support media have been employed in anaerobic filters, including sand, crushed gravel, plastic or ceramic rings and granular activated carbon (*i.e.* Van den Berg, 1984). A good support medium is one to which the bacteria adhere readily and which provides a large surface area for attachment and growth (Young and Dahab, 1983). The fluidized and expanded bed designs overcome the plugging and channeling problems that are

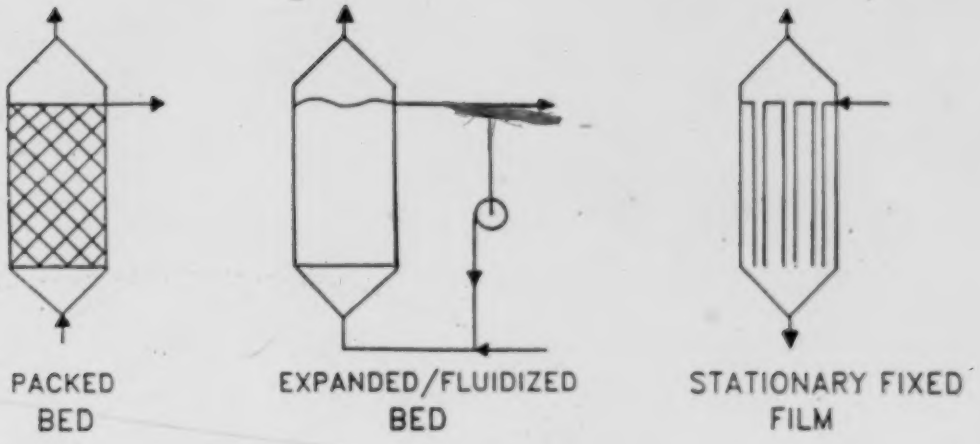


FIGURE 2.2(a): Attached Growth Reactor Designs

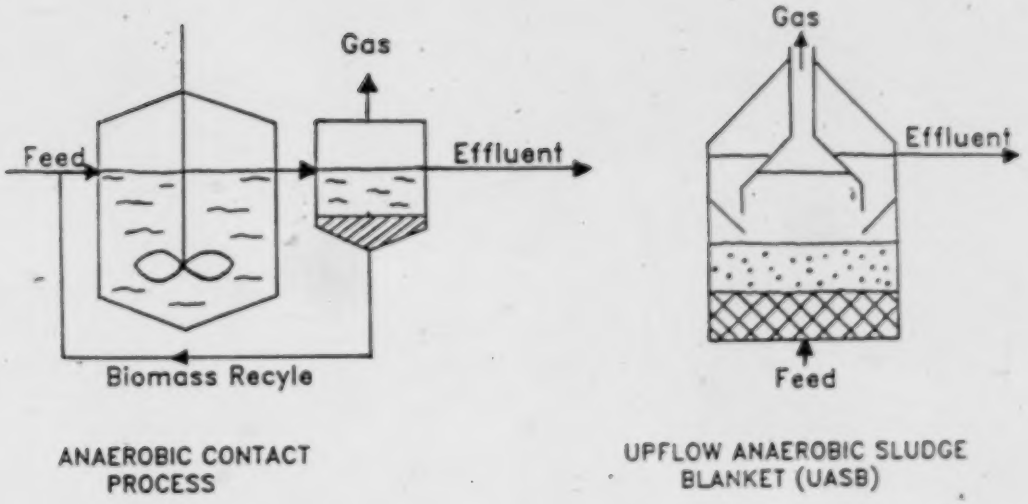


FIGURE 2.2(b): Agglomerated Growth Reactor Designs

often encountered in anaerobic filters and are generally more efficient (Switzenbaum, 1983). Stationary fixed–film reactors employ vertical tubes as the biological support. A continuous biofilm forms on the walls of these tubes.

Reactor designs based on the formation of biomass agglomerates are the anaerobic contact process and the upflow anaerobic sludge blanket (UASB) process, both illustrated in Figure 2.2(b). In the contact process, biomass is separated from the effluent of a well–mixed digester by flocculation and settling. The biomass is then returned to the digester. The contact reactor is effective in treating wastewater with a high content of settlable solids, particularly biodegradable solids, as this tends to promote settling of the bacteria. Originally developed in the Netherlands for the treatment of sugar refinery wastewater (Lettinga *et al.*, 1980), the UASB is an interesting design that has attracted attention for full–scale applications in Europe. The concept is dependent on the formation of a fast–settling, granular biomass. A dense granular layer is established at the bottom of the reactor vessel. Above this layer, a blanket of lighter material forms. Fine, suspended material is quickly washed out of the reactor so that when steady operation is established, the effluent contains very little biomass. An arrangement of baffles in the upper portion of the reactor aids in gas separation and prevents wash–out of biomass particles by flotation. There is no mechanical mixing. Gas production and release in the settled layers provides the required mixing. The granulation phenomenon, upon which this design is based, is discussed in Chapter 3.

Other interesting designs are the hybrid reactors and the two–phase process. Hybrid reactors combine the basic features of the above designs. An example is a UASB reactor that incorporates a packed bed above the sludge bed to increase the efficiency of biomass retention (Lettinga *et al*,, 1983). The principle goal of increasing biomass retention remains the same. In the two–phase process the hydrolytic and acidogenic stage of the degradation pathway is separated from the

acetogenic and methanogenic phases by using two reactor vessels, based on the realization that the metabolic activities of the two major groups of bacteria defined by this separation are more effectively optimized independently (Cohen *et al.*, 1979).

3 LITERATURE REVIEW

The objective of the research project described in this thesis was to study the role of granular activated carbon (GAC) in the enhancement of anaerobic phenol degradation. Literature pertaining to the anaerobic degradation of phenol is reviewed in this chapter. Emphasis is placed on studies in which GAC was employed to improve the treatability of phenolic wastewaters. The formation of granular biomass became important during the course of the experimental program, therefore, a section is dedicated to the subject of granulation in anaerobic treatment systems.

3.1 DEGRADATION OF PHENOL

Application of coal conversion technologies, such as the liquefaction and gasification processes, for the production of synthetic fuels has resulted in an increase in the generation of phenolic wastewaters. The data of Table 3.1, from Fedorak and Hrudey (1984a), describe the phenolic content of a full strength wastewater from a coal liquefaction process. Phenol and the alkyl phenolics (isomers of cresol and dimethylphenol) were the major phenolics present. The dominant organic in the wastewater was phenol, accounting for 62% of the total phenolics and 49% of the organic content in the H –coal effluent. Ammonia and cyanide, potential inhibitors of biological processes, were also present at low levels (6.4 mg/L and 0.21 mg/L respectively).

Phenol and phenolic compounds have long been considered to be highly refractory to biological treatment. Improved culture techniques have enabled the successful anaerobic degradation of some phenolics. Phenol was shown to be converted to methane and carbon dioxide in anaerobic cultures (Healy and Young, 1978). The batch, serum bottle cultures were inoculated with domestic sewage

TABLE 3.1: CONCENTRATION OF VARIOUS PHENOLICS AND VOA'S IN AN H–COAL LIQUEFACTION WASTEWATER[1]

Compound	Concentration(mg/L)[2]
Phenol	4900
o–Cresol	586
m–Cresol	1230
p–Cresol	420
2,4/2,5–Dimethylphenol	63
3,5–Dimethylphenol	213
3,4–Dimethyphenol	44
Total Phenolics	7456
Acetic acid	280
Propionic acid	160
n–Butyric acid	50
n–Valeric acid	35

Notes:

1. information from Fedorak and Hrudey (1984a)
2. determined by gas chromatography

sludge and required a two week acclimation period prior to the onset of phenol degradation and gas production. The stoichiometry for the conversion of phenol to methane and carbon dioxide is given by the general equation for methanogenic degradation of organic compounds developed by Symons and Buswell (Stronach *et al.*, 1986):

$$C_6H_6O + 4\ H_2O \longrightarrow 2.5\ CO_2 + 3.5\ CH_4 \qquad (3.1)$$

Healy and Young (1978) reported methane production at 89% of the theoretical predicted by equation 3.1.

An important consideration in the optimization of phenolic treatment processes is that substrate inhibition kinetics are exhibited at certain concentration levels. Neufeld *et al.* (1980) applied a substrate inhibition model to determine the kinetic parameters of phenol degradation. The maximum specific degradation rate was 0.08 mg phenol/mg VSS/d which is, as the authors pointed out, about 0.5 – 0.8% of the corresponding aerobic degradation rate. VSS refers to volatile suspended solids and is a measure of biomass commonly employed in the biological wastewater treatment field. Inhibition of phenol degradation occurred at phenol concentrations of greater than 686 mg/L. However, the reported growth yield was 0.82 g VSS/g phenol, which is very high compared to typical growth yields for anaerobes (*i.e.* 5%, Sahm, 1984), and no methane production was observed. Because of these drawbacks, the results of the Neufeld *et al.* (1980) study must be interpreted with skepticism. Fedorak and Hrudey (1984b) reported inhibition of methane production at phenol concentrations of greater than 1200 mg/L in batch cultures and presented evidence that the methanogenic bacteria in the phenol–degrading consortium were less susceptible to phenol inhibition than were the phenol–degrading bacteria. They also showed that the presence of other

phenolics can inhibit the degradation of phenol. The effect of some alkyl phenolics on methane production was tested. The only other phenolic, other then phenol, observed to be degraded to methane was *p*–cresol. Dimethyl phenols at concentrations above 500 mg/L were inhibitory to methane production from phenol. Cresols produced the same effect at concentrations above 1000 mg/L. Wang *et al.* (1989) observed complete degradation of phenol at initial concentrations as high as 1400 mg/L in batch cultures, although a reduction in degradation rate was seen at initial concentrations above 600 mg/L. In agreement with the conclusions of Fedorak and Hrudey (1984b), phenol inhibited the action of the phenol–degrading organisms and not that of the methanogens. It should be pointed out that the results of kinetic studies will depend largely on the properties of the inoculum used, *i.e.* type, source and age, as well as culturing procedures and medium composition.

The biochemical pathway catalyzed by the phenol–degrading anaerobic consortium has not been clearly elucidated. Originally it was shown that phenol was reduced to cyclohexanol and then cyclohexanone prior to cleavage of the aromatic ring and further degradation to organic acids (Evans, 1977). Some recent work by Knoll and Winter (1987 and 1989) and Kobayashi *et al.* (1989) has suggested an alternate pathway involving the formation of benzoate in an initial step prior to ring cleavage. Benzoate was detected as an intermediate in phenol degradation (Knoll and Winter, 1987). It is conceivable that both pathways exist and that the one selected in a given situation depends on the nature of the consortia present and the environmental conditions imposed. Figure 3.1, from Knoll and Winter (1987), describes both of these hypothetical pathways.

Even though the complete degradation pathway is uncertain, the consensus in the literature is that acetate is the major intermediate. Knoll and Winter (1987) measured a concentration of acetate as high as 590 mg/L during batch degradation of phenol. This build–up was transient and all of the acetate was eventually

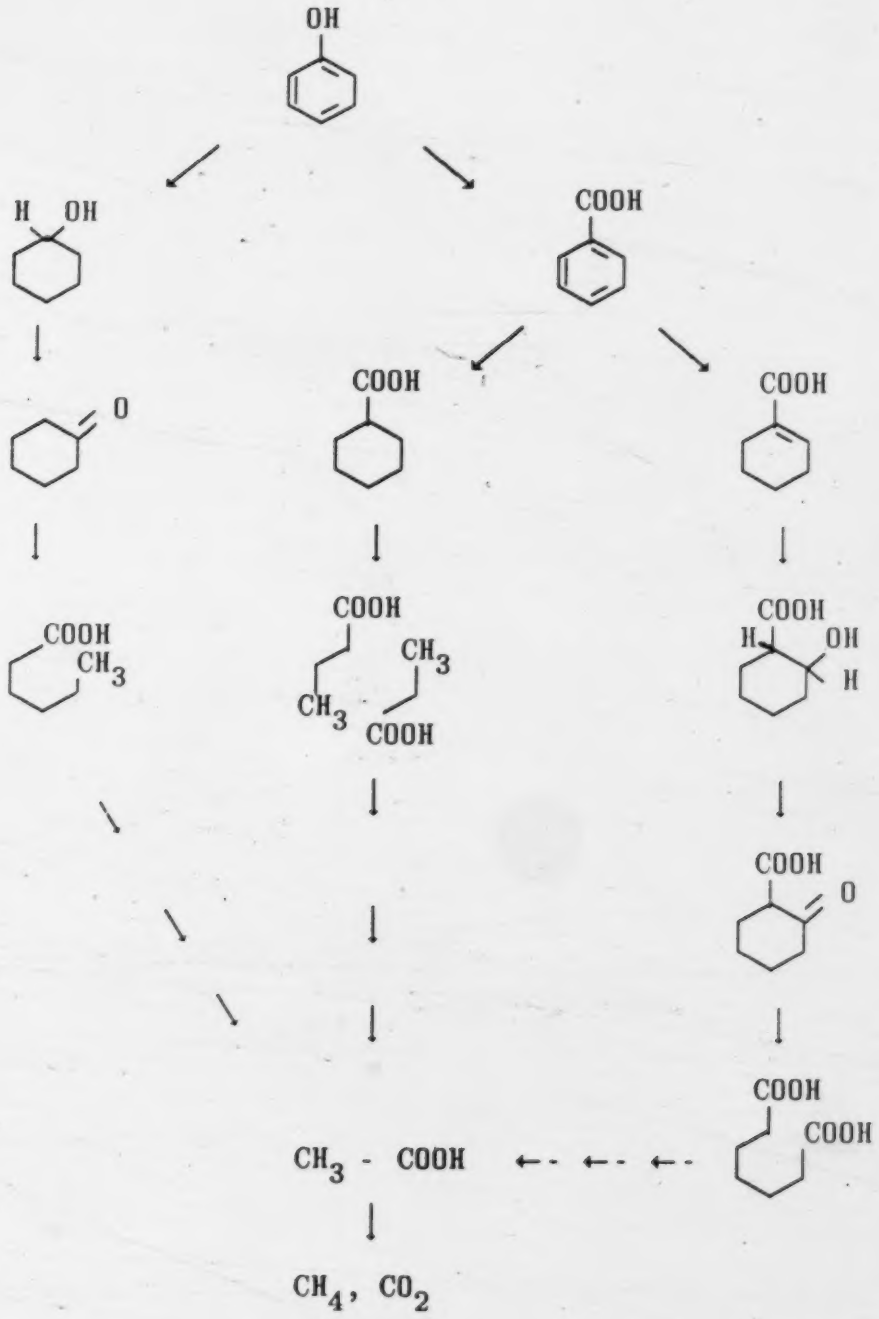


FIGURE 3.1: Proposed pathways for the anaerobic degradation of phenol (Adapted from Knoll and Winter, 1987)

converted to product gases. Wang *et al.* (1989) reported that acetate was the only intermediate which they detected in their experiments, although the levels were much lower at between 10 and 20 mg/L.

A full characterization of the phenol–degrading consortium is also lacking, however, some recent work has produced a partial description of the bacterial types involved. Using scanning electron microscopy, Dwyer *et al.* (1986) distinguished three different morphological types in a methanogenic, phenol–degrading enrichment immobilized in agar. An acetoclastic methanogen was recognized by the filamentous morphology characteristic of the acetate–utilizing *Methanothrix* species. A hydrogen–utilizing methanogen was also identified. A small, oval bacterium was hae proposed phenol–oxidizing organism. Based on this three member consortium and the recognition of acetate as the major intermediate in the pathway, the set of reactions catalyzed by the individual bacterial species is most likely:

$$C_6H_6O_6 + 5\ H_2O \longrightarrow 3\ CH_3COOH + 2\ H_2 \qquad (3.2)$$

$$3\ CH_3COOH \longrightarrow 3\ CH_4 + 3\ CO_2 \qquad (3.3)$$

$$2\ H_2 + 0.5\ CO_2 \longrightarrow 0.5\ CH_4 + H_2O \qquad (3.4)$$

Equation 3.2 is anaerobic oxidation of phenol to acetic acid and hydrogen and equations 3.3 and 3.4 are acetoclastic and hydrogenotrophic methanogenesis, respectively. The sum of these equations yields the stoichiometry for the overall conversion of phenol to product gases predicted by Symons and Buswell (equation 3.1).

Conversion of phenol present in wastewater from a coal conversion process to methane and carbon dioxide has been shown. Methane production was observed in batch anaerobic treatment of volumetric dilutions containing up to 6% of the wastewater in Table 3.1 (Fedorak and Hrudey, 1984a). Unidentified inhibitory

compounds in the wastewater retarded the production of methane from volatile organic acids, indicating that the methanogens were affected. Ether extraction of the wastewater reduced the degree of inhibition substantially. Inhibition by ether–extractable components was not attributed to the major phenolics.

3.2 ROLE OF GAC IN TREATMENT OF PHENOLIC WASTEWATERS

Granular activated carbon has been widely used in both biological and non–biological wastewater treatment processes. The purpose of this section is to review the state of knowledge of the role of GAC in the anaerobic treatment of industrial, phenolic wastewaters. The focus will be on the research work that has aided in establishing that role.

When employed *in situ* in a biological treatment reactor, such as a packed, expanded or fluidized–bed bioreactor, the two main recognized functions of GAC are as follows. Firstly, it serves to increase the microbial retention time by acting as a support medium to which the culture readily attaches. The surface character of GAC, particularly the predominance of surface irregularities, such as macropores and crevices, make it an ideal support medium by providing a large surface area for attachment and growth. These features also provide a protective environment, sheltering the bacteria from the effects of hydraulic shear and inter–particle abrasion. Secondly, by virtue of the adsorption capacity of GAC, it is able to reduce the concentration of toxic and/or inhibitory compounds, which are often present in industrial wastewaters, to levels low enough to permit biological activity. Non–biodegradable constituents may also be removed, thereby making the treatment process more complete. By the same mode, GAC can function as an excellent system buffer by absorbing the effect of sudden surges of inhibitory substrates in the process influent. As discussed in the previous section, above certain levels, phenolic substrates inhibit anaerobic bacteria. At very high levels

they are toxic. Shock loads of these substrates are quickly adsorbed by the GAC preventing the exposure of the bacteria to high aqueous concentrations. Upon returning to normal loading conditions these substrates are slowly desorbed and biodegraded, thus renewing the adsorption capacity of the carbon. This degradation of previously adsorbed material has been given the term bioregeneration.

Several recent studies have investigated these roles in anaerobic bio–treatment of synthetic and authentic, or dilutions of authentic phenolic wastewaters. Both continuous and batch reactor systems have been employed. Table 3.2 gives a chronological listing of these studies.

In anaerobic batch serum bottle experiments, Fedorak *et al.* (1985) showed that the addition of GAC allowed treatment of higher concentrations of wastewater from an H–coal liquefaction process (see Table 3.1). In addition, the acclimation time for the production of methane from the phenolic compounds in the wastewater was shortened. Combinations of H–coal effluent concentrations between 0 and 20% (0 – 1500 mg/L phenolics) and GAC doses from 0 to 2500 mg/L were tested. In the cultures containing low concentrations of both GAC and H–coal wastewater, reduction of the concentration of the major phenolics by adsorption was negligible; however, acclimation times were noticeably reduced due to adsorption of inhibitory materials. At the concentrations experienced in these cultures the major phenolics would not be considered inhibitory. In the cultures containing higher GAC and wastewater concentrations the removal of phenolics by adsorption accounted for further reductions in acclimation times. Adsorbed phenolics were later available for bioregeneration. Batch experiments on the treatability of coal–coking wastewater (Fedorak and Hrudey, 1987) indicated severe inhibition of methanogenesis. Addition of GAC only partially relieved the inhibitory effects of the wastewater. The identity of the inhibitory substance was not confirmed, however, cyanide, which was present in the wastewater and which is not strongly adsorbed, was

TABLE 3.2: STUDIES EMPLOYING GRANULAR ACTIVATED CARBON

INVESTIGATOR	SYSTEM	WASTEWATER
Suidan *et al.* (1981)	GAC PBR	synth. catechol, o–cresol
Khan *et al.* (1981)	GAC PBR	synth. phenol, glucose
Khan *et al.* (1982)	GAC PBR, coal PBR	synth. phenol, glucose, auth. aircraft paint stripping–bath
Cross *et al.* (1982)	RR PBR + GAC PBR	dil. auth. CCWW
Suidan *et al.* (1983a)	BS PBR + GAC EBR + aerobic nitrification	synth. CCWW
Suidan *et al.* (1983b)	same as 1983a	dil. auth. CCWW
Harper *et al.* (1984)	GAC FBR	dil. auth. CCWW
Fedorak and Hrudey (1985)	Batch serum bottles	dil. auth. CCWW
Kim *et al.* (1986)	RR PBR + GAC FBR	synth. phenol
Wang *et al.* (1986)	GAC EBR	synth. phenol
Suidan *et al.* (1987)	same as 1983a	synth. CCWW
Fedorak and Hrudey (1987)	Batch serum bottles	dil. coal–coking wastewater
Fox *et al.* (1988)	BS PBR + GAC EBR	synth. CCWW
Gardner *et al.* (1988)	GAC FBR, NAC FBR	Refinery sour–water stripper bottoms
Pfeffer and Suidan (1989)	GAC FBR	dil auth. CCWW
Kindzierski (1989)	Sequential–batch serum bottles	synth. phenol

DEFINITIONS OF SYMBOLS FOR TABLE 3.2

PBR	.....	Packed bed reactor
EBR	.....	Expanded bed reactor
FBR	.....	Fluidized bed reactor
BS	.....	Berl saddles
RR	.....	Rashig rings
GAC	.....	Granular activated carbon
NAC	.....	Non–activated carbon
CCWW	.....	Coal conversion wastewater
synth.	.....	synthetic
auth.	.....	authentic
dil.	.....	volume dilution

suspected.

Suidan *et al.* (1981) successfully treated a synthetic, catechol–containing wastewater using two GAC packed–bed reactors in series. Catechol removals of greater then 99% were achieved at steady–state on a feed of 1000 mg/L catechol. Based on volumetric loading, biological conversion rates were comparable to those achieved in aerobic systems. In the same study, no degradation of *o*–cresol was observed. Using a similar system, Khan *et al.* (1981) achieved high removals of phenol and glucose. These and similar studies from Table 3.2 exhibited the same reactor start–up characteristics. In an initial phase, no biodegradation of phenolics occurred and removal was attributed almost exclusively to adsorption. With the onset of breakthrough, phenolic substrate was made available to the bacteria and methanogenic activity commenced. Bioregeneration was then observed when methane production exceeded the theoretical maximum attributable to the biodegradation of the organics in the feed. Methane production increased until a steady–state was achieved. At this point of operation, biological breakdown was the main means of phenolic removal. Wang *et al.* (1986) used partially saturated GAC to eliminate the initial adsorption phase in treatment of a synthetic, phenol–containing wastewater. They also detected a loss of attached biomass at higher loading rates which they attributed to increased gas shearing.

In attempts to determine the relative importance of the mechanisms which account for the effectiveness of GAC in anaerobic bio–treatment, several investigators carried out studies in which the performance of GAC was compared to that of other media. Khan *et al.* (1982) demonstrated the superior performance of GAC over non–porous anthracite coal in terms of organic removal, methane production and biomass retention for a system treating both a synthetic, phenol bearing wastewater and a diluted aircraft paint stripping–bath wastewater. The authors suggested substrate polarization and the ability of the GAC to shelter dense

bacterial populations as mechanisms for improved performance. The GAC system readily absorbed a surge in feed phenol concentration after 275 days of continuous operation, demonstrating the buffering capacity of the system. Suidan *et al.* (1983a) employed a system consisting of a reactor packed with Berl saddles followed by a reactor containing an expanded bed of GAC to treat a synthetic analog of a coal gasification wastewater containing a complex mixture of phenolics and known inhibitors (*i.e.* cyanide, ammonia, thiocyanate). This treatment was followed by an aerobic nitrification step. There was very little removal of organics in the Berl saddle reactor although removal efficiencies in the GAC reactor were high. An identical system was used to treat a solution containing 10% authentic coal gasification wastewater (Suidan *et al.*, 1983b), with similar results. The low biological activity in the Berl saddle reactor is suggestive of the special role that GAC plays in adsorption of toxic and inhibitory material. Cross *et al.* (1982) observed the same low bio–activity in a Rashig ring packed reactor treating a dilution of coal conversion wastewater. In a later study, Suidan *et al.* (1987) noticed methane production from glucose in a Berl saddle reactor even in the presence of a synthetic coal conversion wastewater, suggesting that the inhibitive constituents of the wastewater affect the bacteria that perform the initial breakdown of the aromatic ring, rather then the methanogens. This observation is contrary to observations made by Fedorak and Hrudey (1987) in treating coal coking wastewater. Gardiner *et al.* (1988) compared the performance of non–activated carbon to GAC in treating refinery sour–water stripper bottoms in a fluidized–bed. They also investigated the effect of varying GAC particle size. The poor performance of the non–activated carbon system relative to the GAC system reflects the necessity of toxicity removal by adsorption. The superior performance of the smaller GAC particles observed at higher wastewater loading rates was attributed to increased surface area for microbial growth and decreased mass

transfer resistance during adsorption.

These studies were able to demonstrate quite clearly that the adsorption capacity of activated carbon was crucial in reducing the level of toxic compounds in order to permit biological treatment. It was realized, however, that the adsorption capacity of the GAC for toxic and/or inhibitory constituents of coal conversion wastewaters was limited and would eventually become exhausted. This need was first identified by Harper *et al.* (1984) in a study in which a solution containing 10 – 12% coal conversion wastewater was treated in a fluidized–bed reactor. Shortly after saturation of the GAC by cresols, reactor performance declined sharply indicated by a decrease in COD removal efficiency and cessation of gas production. An accumulation of volatile acids was observed, suggesting that the methanogens were inhibited first. Performance improved immediately after replacement of 10% of the carbon with fresh material. To maintain performance levels for the longer term, subsequent carbon replacements were required. At the end of the study the authors suggested an optimum steady–state carbon replacement schedule of 0.6 – 0.8 kg GAC per kg of organic carbon fed to the system, noting that removal of too large a portion of the GAC results in excessive loss of biomass. Suidan *et al.* (1987), Fox *et al.* (1988) and Pfeffer and Suidan (1989) also reported the requirement of GAC replacement in the systems they were studying.

Kindzierski (1989) carried out a study with the objective of identifying the features of a biological support that account for enhanced removal and eventual conversion of phenol to methane. Properties of three different potential supports (Filtrasorb F300 activated carbon, Dowex MSC–1 cation exchange resin and Ambergard XE–352 anion exchange resin) which determined their abilities to function as microbial supports were characterized and compared. These included surface chemistry, pore volume and surface area available for microbial attachment and proliferation, and phenol adsorptive capacity. Initial bacterial affinity for the

supports was determined by adsorption studies with radiolabelled cells. The actual performance of the supports was determined in sequential–batch serum bottle experiments which employed a model wastewater containing phenol as the carbon source and the necessary nutrients for growth. Scanning electron microscopy enabled a qualitative description of the attached culture characteristics. Since the model wastewater did not contain the toxic and inhibitory compounds prevalent in an authentic wastewater, the study was able to provide further insight into those characteristics, aside from adsorption capacity, that enable a material to function as an effective microbial support.

Pore capacity and associated surface area were determined to be the most important features that encourage attachment and growth of biomass on the supports. Based on pore–size distribution and a knowledge of bacterial sizes, the internal surface area available for attachment and growth was estimated to be 0.0173 m^2/g for the F300 activated carbon, 0.227 m^2/g for the XE–352 resin and close to zero for the MSC–1 resin. Cell adsorption studies showed that the XE–352 resin possessed a higher initial affinity to *Pseudomonas aeruginosa* cells in suspension than did the F300 activated carbon. The MSC–1 resin displayed negligible adsorption affinity. Results of the phenol bio–activity assays showed that there was no increase in surface attached bio–activity of the F300 activated carbon after 75 days of culturing. The supernatant, however, possessed a significant amount of activity suggesting that the activated carbon had become overloaded biological growth. Continued growth on the surface was being offset by removal mechanisms such as hydraulic shear and inter–particle abrasion. Even after 88 days the bio–activity of the XE–352 supported culture continued to increase and the activity in the supernatant was far less then that which was observed in the activated carbon cultures. There was negligible bio–activity associated with the MSC–1 resin. Scanning electron microscopy revealed extensive attachment and

growth of the bacteria inside the surface pores and crevices of the activated carbon and the pores and internal areas of the XE–352 resin. Very little attachment was observed on the exposed outer surface of any of the supports. Because the MSC–1 resin did not possess a porous structure or an irregular surface no growth was fostered on this support. The large surface pores of the activated carbon and the XE–352 resin offer an environment where the bacteria are protected from the effects of hydraulic shear and inter–particle abrasion. Response to pulses of high phenol concentration showed that the suspended bacteria from the XE–352 resin culture were more susceptible to substrate inhibition then were the attached bacteria. The phenol adsorption capacity of the resin was negligible suggesting that the protection of the attached bacteria from the inhibitory concentration of phenol in the bulk liquid could be attributed to mass transfer limitations within the immobilized culture.

Of particular interest in the Kindzierski study was the observation that the biomass–loaded activated carbon was capable of rapidly transferring bio–activity into the liquid medium. Some results of sequential–batch degradation experiments which illustrate the re–seeding of the supernatant are given in Figures 3.2(a) and 3.2(b). After 75 days of growth on the GAC a sequence of supernatant withdrawals and replacements was commenced. In this procedure, 88% of the supernatant liquid was withdrawn from the culture and replaced with cell–free medium. This quantity was the largest aliquot of liquid which could be withdrawn without removing a portion of the carbon particles. The removed supernatant was divided into two fractions. A 12% fraction represented the portion which remained behind with the activated carbon. The second fraction was the remaining 76%. Each of the three cultures, the activated carbon culture and the 12% and 76% supernatant cultures, was fed the same amount of phenol. Figure 3.2(a) shows the decrease in phenol concentration in the supernatant of each of three cultures with time until there is no

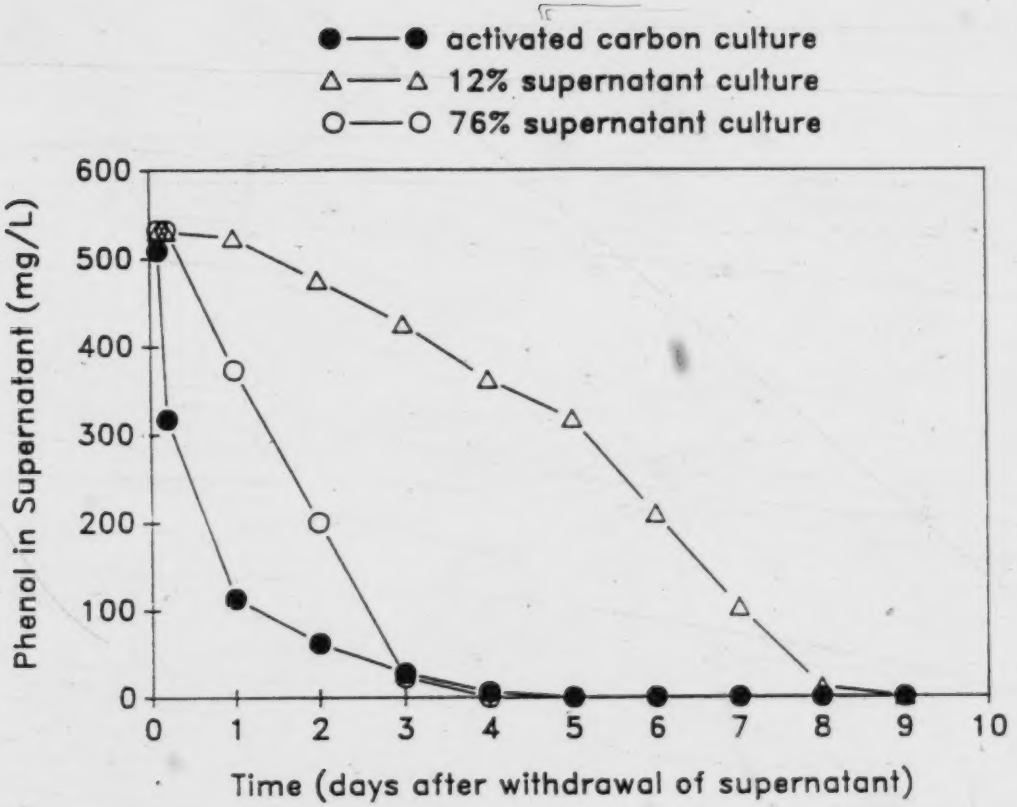


FIGURE 3.2(a): **Phenol biodegradation in GAC–containing sequential–batch cultures after the first of a series of supernatant withdrawals and replacements (data from Kindzierski, 1989)**

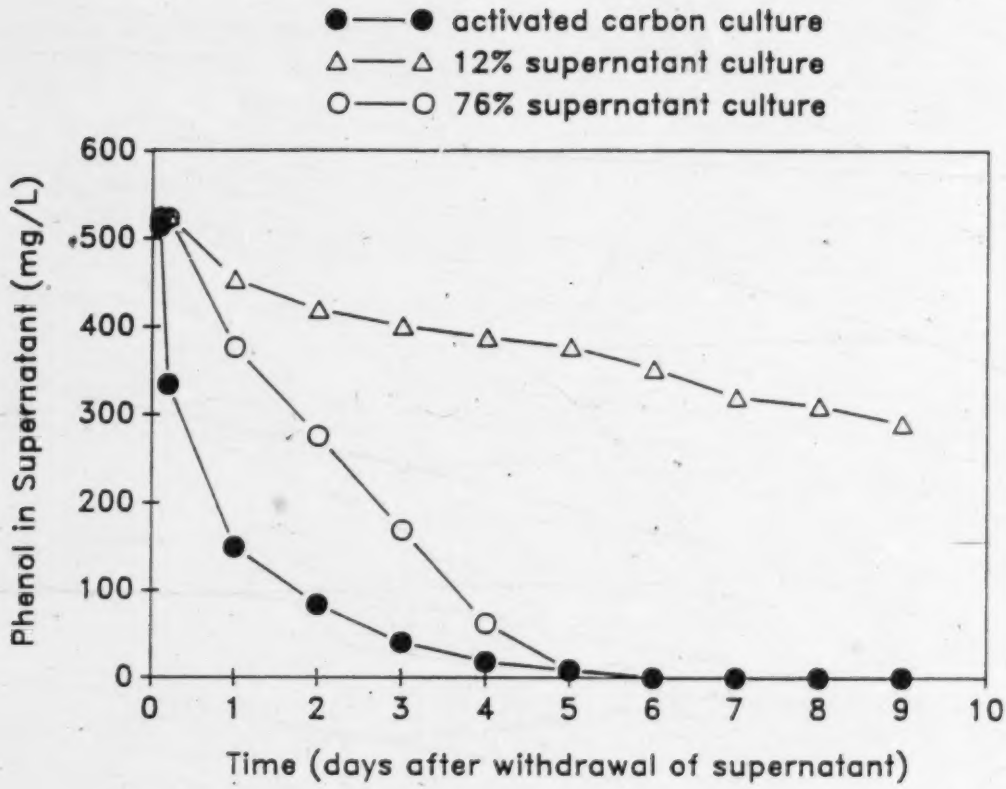


FIGURE 3.2(b): Phenol biodegradation in GAC–containing sequential–batch cultures after the fourth of a series of supernatant withdrawals and replacements (data from Kindzierski, 1989)

phenol remaining. Note the initial rapid decrease of phenol concentration in the activated carbon culture which was due to adsorption. This phenol was subsequently desorbed from the activated carbon and biodegraded. The 76% supernatant culture possessed a significant amount of phenol–degrading activity relative to the activated carbon–containing culture. The procedure was repeated sequentially and the results of the fourth supernatant withdrawal and replacement are presented in Figure 3.2(b). As the figure shows, the 76% supernatant still possessed significant phenol–degrading activity although to a lesser degree than in the first supernatant withdrawal. The activity of the activated carbon–containing culture was approximately the same. This suggests that the activated carbon–containing culture was rapidly transferring bio–activity to the supernatant liquid in the form of new growth. If it was not, the activity of the supernatant would have decreased by 98% by the fourth withdrawal because of the sequential dilution procedure.

The previous work has, therefore, been successful in partially characterizing the role GAC in enhancing anaerobic treatment of phenolic wastewaters. The importance of the adsorption capacity of GAC in treating authentic wastes has been unequivocally established. Kindzierski's work provided new information on the nature of GAC as a microbial support. Under the hydraulic conditions experienced in constantly shaken serum bottles, the colonization of the GAC eventually reached a maximum after which any further growth was transferred into the supernatant. This re–seeding of the liquid medium could have significant implications in treatment reactor design. None of the literature mentioned in this review has reported the specific phenol degradation rate of bacteria immobilized on GAC. The difficulties in determining the phenol degradation kinetics in GAC systems are as follows:

1. The effects of adsorption and desorption of phenol on the apparent

degradation rates cannot be easily assessed. Kindzierski (1989) demonstrated the effect of bacterial growth on the phenol adsorption kinetics. The rate of uptake and adsorption capacity were both noticeably reduced, presumably as a result of blockage of the macropores with biomass as illustrated by Figure 3.3.

2. The determination of specific degradation rates requires an estimate of the quantity of active biomass. No satisfactory method for directly measuring the quantity of biomass attached to the GAC in anaerobic system is available.

3. Biological activity is a function of many variables and wastewater treatment systems are extremely complex. The selection of an appropriate set of control conditions is difficult.

Serum bottle studies suffer from an additional disadvantage in evaluating the performance of attached growth systems in that the environment of hydraulic shear and inter–particle abrasion is not representative of the environment that exists in actual treatment reactor configurations such as the packed, expanded or fluidized–bed reactors.

3.3 GRANULATION IN ANAEROBIC TREATMENT REACTORS

Development of biomass granules with good settling properties and high specific activities is an excellent method of increasing bacterial retention times in treatment reactors. No inert support medium is required, therefore the inactive fraction of the reactor volume is reduced. This phenomenon of biomass granulation has the potential to influence greatly the design of advanced treatment reactors. The mechanisms involved in the initiation and promotion of bacterial granulation, however, appear to be quite complex and are far from being fully understood. The aim of this section is to provide a brief review of the current understanding of the

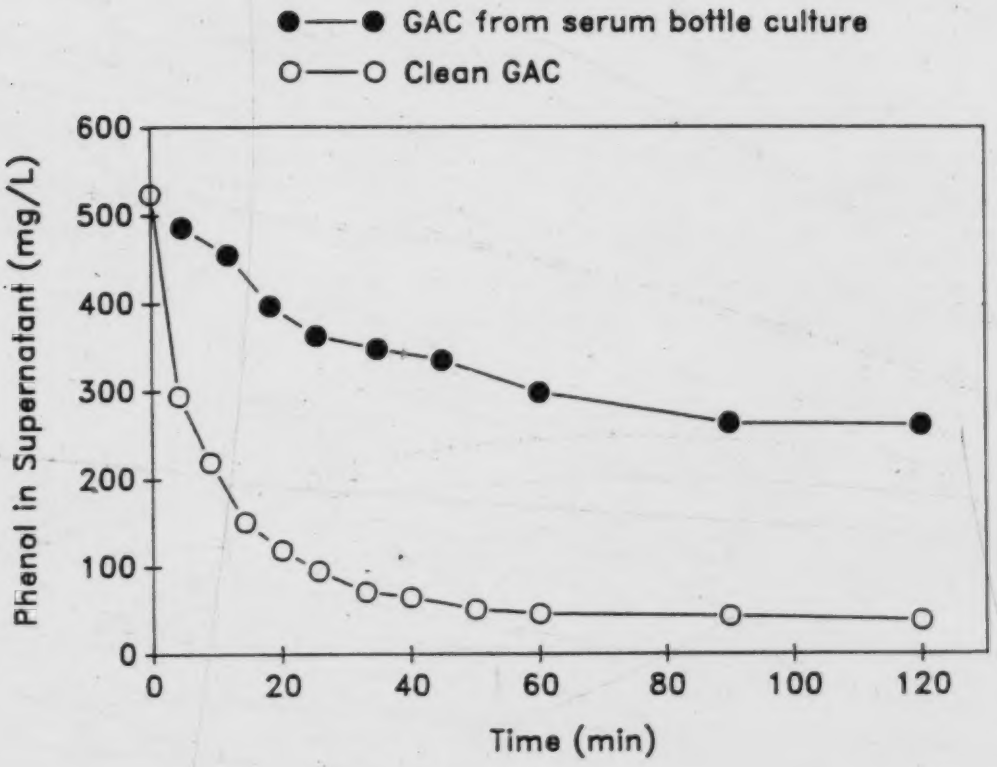


FIGURE 3.3: **Phenol adsorption onto clean GAC (without bacteria) and GAC from serum bottle cultures (in the presence of attached growth) (data from Kindzierski, 1989)**

granulation phenomena in anaerobic treatment systems.

Interest in biomass granulation in anaerobic treatment was initiated by the development of the upflow anaerobic sludge blanket (UASB) reactor. As reported by Lettinga *et al.* (1980 and 1983), the formation of dense layers of flocculated and granular biomass is integral to the UASB design concept. The distinction between granules and the bacterial flocs which are generally observed in anaerobic treatment processes can be considered as follows. Flocculation occurs when cells in suspension form conglomerations having relatively loose structures, or flocs. Upon settling together, the flocs form a uniform layer of biomass and are no longer individually distinguishable. Granules are more dense and particulate in appearance and remain visible as separate entities after settling. They also have significantly higher settling velocities.

Many factors appear to be involved in the granulation process. Hulsoff Pol *et al.* (1983) summarize factors that are considered to be important in the development of granular biomass in UASB reactors as follows:

1. environmental conditions such as like wastewater composition, the availability of nutrients, the concentration of univalent and divalent cations, the presence of inhibitory compounds and finely dispersed non–biodegradable matter, temperature, pH, etc.,
2. the type of inoculum (*i.e.* source, composition, specific activity),
3. the process conditions applied during start–up such as like organic loading rate and quantity of inoculum used.

Hulshoff Pol *et al.* (1983) carried out an experimental study with the objective of evaluating the impact of several of these factors on the development of granules. They discovered that wastewater composition and start–up procedures play a dominant role. The addition of a small amount of granular biomass with the inoculum did not have a significant effect on granule formation. A selection process

was observed to take place during the initial reactor start–up phase in which the smaller, suspended biomass particles were washed out with the effluent. The larger, granular particles were retained and growth was subsequently concentrated on this granular biomass. When returned to the reactor, the suspended material was not able to form granules and was soon washed out again. Flotation of flocculated and granular biomass, resulting in wash–out, occurred due to the increased gas production and retention of gas bubbles within the particles which accompanied higher organic loadings. An optimal concentration of Ca^{2+} for granule promotion was suggested by their findings. Divalent cations are known to exert a positive influence on the flocculation of anaerobic biomass. Using mixtures of acetate, propionate and butyrate as substrates, two distinct types of granules were observed to develop in separate reactors. The filamentous granules were composed mainly of long, multicellular filaments of rod–shaped organisms resembling the acetate–utilizing methanogen, *Methanothrix soehngeii*. Rod–type granules also consisted of *Methanothrix* species but as short multicellular fragments. The filamentous granules all contained some kind of inert material which originated with the inoculum. Filamentous and rod–shaped granules of up to 2 mm and 5 mm in size, respectively, were observed and the average settling velocity of both types of granules was
0.5 m/min.

Granulation has been observed to occur readily in methanogenic thermophilic systems as well as in mesophilic systems. Wiegant and Man (1986) observed similar *Methanothrix* granule types in a study of thermophilic granulation of acetate–degrading anaerobes. They were also able to cultivate *Methanosarcina* granules by applying a selection pressure, but it was discovered that these granules were more readily washed out of the system. Addition of inert support media and the type of inoculum did not effect the formation of *Methanothrix* granules. The

authors noted, however, that in another study, on mesophilic digestion, addition of hydroanthracite particles did significantly reduce the time required for granulation to be observed.

The chemical composition of various bacterial granules grown on sugar refinery wastewater and synthetic volatile–acid mixtures was reported by Dolfing *et al.* (1985). The protein, carbohydrate, total organic carbon and nitrogen contents were found to be comparable to that of bacteria in general. The ash content was somewhat higher, suggesting that inert organic material was incorporated into the particle matrix. Scanning and transmission electron microscopy indicated the presence of significant extracellular polymer material in the matrix. Secretion of a polysaccharide glycocalyx has been suggested as a mechanism by which bacteria bind to each other to form flocs or granules. Extracellular sugars accounted for 1 – 2% of the dry weight of the granules. According to Stokes Law, the settling velocity of a particle is directly proportional to the particle size and the effective particle density. The effective density of the granules was determined to be 1.00 – 1.05 g/cm^3 which is approximately equal to that of dispersed cells. This suggests that the observed settling properties of the granules were mainly a result of an increase in particle size reulstinf from simple agglomeration of the bacteria.

In a densely packed granule, significant resistance to the diffusion of substrate into the particle interior might be encountered. In particles of sufficient size, mass transfer limitations can be expected to reduce the efficiency of substrate conversion. Dolfing *et al.* (1985) showed that the effect of mass transfer limitations on the kinetic ability of methanogenic granules is dependant on the specific activity of the bacteria within the granule and the thickness of the bio–layers of which the granules are composed. Mass–transfer limitations were found to be significant at low substrate concentrations and in large granules with high methanogenic activity. No mass transfer limitations were detected for the degradation of volatile organic

acids in experiments with granules from an industrial reactor. The granules were less than or equal to 2 mm in size. The methanogenic activity of bacteria degrading a synthetically prepared medium containing a simple carbon source, such as organic acids, and all the appropriate nutrients for growth and metabolism will be greater than what would be achieved in a treatment reactor treating a complex, real wastewater. The author of the study concluded, therefore, that mass transfer limitations will not be of much influence in industrial reactors.

Although experimental studies, such as those mentioned above, have provided some useful information on the granulation process, there are still many unanswered questions. For instance, mechanisms responsible for for the initiation of granule formation are not known. The granulation process is most likely influenced by several different factors acting simultaneously. Characterization of granules grown under certain specific conditions is possible in these types of studies, however, the results are specific to the system. It is difficult, if not impossible, to elucidate general mechanisms and draw fundamental conclusions. A generalized procedure for optimization of granule growth is not available. Studies so far have involved a limited range of wastewater substrates (*i.e.* organic acids, sugar refinery wastewaters), even though the granulation process is a strong function of wastewater composition.

4 EXPERIMENTAL METHODS

4.1 ANAEROBIC CULTURE TECHNIQUES

Maintenance of strict anaerobic conditions was required during all operations involving the phenol–degrading anaerobes. Procedures employed for growing the phenol–degraders were based on the principles of anaerobic technique developed by Hungate (1969). All vessels, containers, fittings, tubing and attachments were thoroughly flushed with a gas mixture of 30% CO_2 in N_2, prior to the addition of medium or bacteria. Oxygen was removed from the gas mixture by passing it through a glass column containing a copper heating coil. Before each use, the heated copper coil was reduced by passing a mixture of 5% H_2 in N_2 through the column for 15 min. Syringes and needles employed for the transfer of gases or liquids into or out of the culture systems were rinsed several times with the 30% CO_2 in N_2 gas mixture before use.

4.2 CULTURE MEDIUM

The complete culture medium used by Fedorak and Hrudey (1986) was used in the present study. The medium was composed of stock solutions containing components necessary for growth *i.e.* phosphate, cation salts, trace metals and B vitamins. Buffering of the pH was provided by the addition of bicarbonate. Sodium sulphide was added as a reducing agent and resazurin served as a redox indicator. The compositions of the stock solutions are given in Table 4.1. The final medium composition, without phenol, is given in Table 4.2.

Medium was prepared by combining the stock solutions with double–distilled water (dd H_2O) in the proportions listed in Table 4.2 in an appropriate size Erlenmeyer flask. 3.5 L of medium were prepared in a 4–L flask for reactor feed and 800 mL were prepared in a 1–L flask for other purposes. Boiling chips were added

TABLE 4.1: COMPOSITION OF STOCK SOLUTIONS USED IN CULTURE MEDIUM

SOLUTION	COMPOUND	CONCENTRATION IN dd H_2O (g/L)
Mineral Solution I[1]	NaCl	50
	NH_4Cl	50
	$CaCl_2 \cdot 2H_2O$	10
	$MgCl_2 \cdot 6H_2O$	10
Mineral Solution II	$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	10
	$ZnSO_4 \cdot 7H_2O$	0.1
	H_3BO_3	0.3
	$FeCl_2 \cdot 4H_2O$	1.5
	$CoCl_2 \cdot 6H_2O$	10.0
	$MnCl_2 \cdot 4H_2O$	0.03
	$NiCl_2 \cdot 6H_2O$	0.03
	$AlK(SO_4)_2 \cdot 12H_2O$	0.1
Vitamin B Solution	Nicotinic acid	0.1
	Cyanobalamine	0.1
	Thiamine	0.05
	p–Aminobenzoic acid	0.05
	Pyridoxine	0.25
	Pantothenic Acid	0.025
Phosphate Solution	KH_2PO_4	50
Resazurin Solution		0.1
Sodium Sulphide[2]	$Na_2S \cdot 9H_2O$	25

Notes:

1. dissolved in 0.01 M HCl rather than dd H_2O

2. prepared with boiled dd H_2O and sealed in gassed serum bottles

TABLE 4.2: COMPOSITION OF COMPLETE CULTURE MEDIUM[1]

COMPONENT	AMOUNT
Mineral Solution I	1.0 mL
Mineral Solution II	0.1 mL
Vitamin B Solution	0.1 mL
Phosphate Solution	1.0 mL
Sodium Bicarbonate	0.57 g
Double Distilled Water	97.0 mL
Resazurin Solution	1.0 mL
Sodium Sulphide Solution	1.0 mL

Notes:

1. no phenol in this medium

and the contents of the flask were brought to a full boil which was maintained for two minutes in order to remove dissolved O_2. The medium was then cooled under a stream of O_2–free 30% CO_2 in N_2 by immersing the flask in an ice–water bath. After cooling to room temperature, the sodium bicarbonate was added to the flask. Bubbling of gas through the medium was continued until the pH was between 6.9 and 7.1. Final reduction of the medium was accomplished by addition of 10 mL of 2.5% Na_2S solution per 1 L of complete medium.

4.3 SERUM BOTTLE PROCEDURES

Before addition of culture and/or medium, the serum bottles were flushed with 30% CO_2 in N_2 gas. After the addition, the headspace of the bottles were flushed for a few more minutes. The bottles were then sealed with butyl rubber stoppers which were secured in place with crimped aluminum caps. The serum bottle contents were then reduced by addition of 2.5% Na_2S solution. Additions of concentrated phenol feed solution or Na_2S solution were made using plastic syringes fitted with 22–gauge needles.

Stock solutions of concentrated phenol feed were prepared for use in batch feedings of the bioreactor and the serum bottle cultures. The required quantity of phenol was accurately weighed into 158–mL serum bottles. The bottles were flushed with O_2–free 30% CO_2 in N_2 gas for 20 min. While flushing of the headspace continued, exactly 100 mL of boiled and pH–adjusted medium was transferred anaerobically from the preparation flask into the serum bottles in pre–flushed 25–mL pipettes. The headspace of the bottles was flushed for a few more minutes and then the bottles were stoppered and capped. The contents were then reduced by addition of 1 mL of 2.5% Na_2S solution.

4.4 PHENOL ANALYSIS

Phenol concentrations of liquid samples were determined on a Hewlett–Packard model 5700A gas chromatograph. The GC was equipped with a flame ionization detector and a 2 m x 2 mm I.D. stainless steel column packed with 5% polyphenylether (Chromatographic Specialties) coated 60/80 mesh Tenax GC packing (Alltech Assoc.). Helium (30 mL/min) was the carrier gas. Hydrogen (40 mL/min) and air (300 mL/min) were supplied to the flame ionization detector. Oven, injector and detector temperatures were 200°C, 250°C and 250°C respectively. Peak areas were obtained from a Hewlett–Packard model 3390A integrator.

Suspended solids were allowed to settle in the liquid samples withdrawn from the bioreactor. Using a 10–μL Hamilton syringe, 3 μL of the supernatant was were injected into the GC and the peak area was determined. Sampling of the serum bottle cultures was accomplished by first inverting the bottles and allowing the bulk of the suspended solids to settle and then inserting the needle of a 10–μL Hamilton syringe through the stopper and withdrawing a portion the supernatant. In the case of samples with high phenol concentrations, (*e.g.* 500 mg/L – 2000 mg/L), the sample was first volumetrically diluted with dd H_2O, using an Eppendorf micropipette and/or a 10–μL Hamilton syringe, to reduce the concentration to between 100 and 500 mg/L. Three microlitres of the resulting dilution were injected into the GC and the peak area determined.

Peak areas were compared to calibration curves to determine the phenol concentration. Calibration curves were determined by linear regression analysis of the peak areas resulting from injections of a series of standard solutions with concentrations spanning the range of the sample concentrations. The standards were carefully prepared in volumetric glassware and the concentrations were accurately known. A new calibration curve was determined following any

disturbances in the carrier or detector gas flowrates or after re–lighting of the detector flame. Injections of standards were carried out in a manner identical to injection of samples. All injections of samples and standards were performed at least 3 times and the average peak area was determined.

4.5 ACETIC ACID ANALYSIS

Acetic acid concentration was determined on a HP 5790 GC equipped with a 2 m x 2 mm I.D. glass column packed with GP 10% SP1000/1%H_3PO_4 on 100/200 Chromosorb WAW (Supelco) and a flame ionization detector. Helium (20 mL/min) was the carrier gas and air (300 mL/min) and hydrogen (30 mL/min) were supplied to the flame ionization detector. Oven, injector and detector temperatures were 130°C, 200°C and 250°C respectively. Peak areas were obtained using a HP model 3390A integrator.

Supernatant of liquid samples from the bioreactor was acidified by combining 100 μL of the supernatant with 20 μL of 4 N phosphoric acid solution. Prior to injection of sample, the GC column was also conditioned by injecting 1.0 μL of 0.1% formic acid solution. One microlitre of sample was injected into the GC using a 5–μL Hamilton syringe. A calibration curve was determined each day samples were run. Quantitatively prepared standard solutions, spanning the range of the sample data, were acidified and injected in the same manner as the samples. All injections of samples or standards were performed at least three times and the average peak area was determined.

4.6 METHANE ANALYSIS

The concentration of methane in the headspace of the bioreactor was determined on a Microtek GC equipped with a flame ionization detector and a 2 m x 2 m I.D. glass column packed with Chromosorb WAW (Supelco). Nitrogen

(20 mL/min) was the carrier gas. Air (300 mL/min) and hydrogen (30 mL/min) were supplied to the detector. The oven, injector and detector were maintained at ambient temperature. Samples (0.1–mL) were injected into the GC with Lo–dose gas tight syringes (Becton Dickinson). Peak areas were recorded on an HP3390A integrator.

Peak areas were converted into concentrations by comparing the data to a calibration curve consisting of 3 points; 0, 29.9 and 100% methane. Standards were prepared by filling 158–mL serum bottles with 30% CO_2 in N_2, 29.9% methane and 100% methane gases from prepared gas cylinders and then sealing the bottles with rubber septa. A calibration curve was prepared each day samples were run. The average peak areas were determined for three injections of standard and five injections of headspace gas. The methane concentration of the headspace gas determined from the GC analysis was corrected for moisture content by assuming saturation at the reactor temperature (*i.e.* 35 °C). This determination of methane concentration was not intended to be highly precise and was only used as a check on methanogenic activity and in the approximate of the reactor phenol balance.

4.7 SCANNING ELECTRON MICROSCOPY

Scanning electron microscopy was used to view samples of the the colonized GAC and flocculated biomass from the bioreactor. Edith Schwalt in the Faculty of Dentistry at the University of Alberta, Edmonton, Alberta performed the entire S.E.M. procedure, including sample preparation. Kindzierski (1989) described the sample preparation procedure:

1. Support materials were fixed overnight in 2.5% glutaraldehyde in Milloning's Buffer.
2. Samples were rinsed 3 times (15 min each) with fresh Milloning's buffer.

3. Samples were dehydrated in a series of ethanol solutions for 15 min each (50, 70, 80, 90% ethanol solutions)
4. Samples were dehydrated 3 times (10 min each) in fresh 100% ethanol.
5. Samples were critical–point dried in a See Vac Inc. Critical Point Dryer .(Florida, U.S.) with liquid CO_2.
6. Samples were mounted on aluminum stubs with silver conductive glue and dried overnight in a desiccator.
7. Samples were sputtered with gold (75–150 nm thickness) in an Edwards Sputter Coater (Model S150B)
8. Samples were examined and photographed in a Philips SEM (Model 505).

4.8 VOLATILE SUSPENDED SOLIDS

Volatile suspended solids (VSS) concentration was determined by a standard method (APHA, 1985, Section 208). A glass microfibre filter (Whatman GF/C, 4.25 cm) was placed in an aluminum dish, dried for 15 min in a 550°C air oven, and cooled to room temperature in a desiccator. The weight of the filter and dish were determined to the nearest 0.1 mg. Samples containing settlable biomass, *i.e.* biomass flocs, were well shaken to suspend the biomass immediately before filtering. A precise volume of a liquid sample containing biomass solids was filtered through the pre–wetted filter on a suction flask apparatus. The filtered solids were washed with 50 mL of dd H_2O. The filter was then carefully returned to the aluminum dish and dried at 105°C for 1 h. The dish and filter were cooled in a desiccator, weighed and then fired in a 550°C oven for 15 min. The cooling and weighing was repeated and the weight loss was determined. Total suspended solids (TSS) concentration was determined along with the VSS. Triplicate determinations were performed and the average was reported.

4.9 THERMOGRAVIMETRIC ANALYSIS

Thermogravimetric analysis was used to estimate the quantity of biomass immobilized on the GAC particles in the bioreactor. In thermogravimetric analysis, the weight of a small (mg size) sample of material is continuously and accurately monitored while the sample is subjected to a desired temperature environment. The weight gain or loss of the sample as a function of temperature is determined.

A Perkin Elmer TGS–2 Thermogravimetric System (model no. 319–0272) combined with a Perkin Elmer System 4 Microprocessor Controller (model no. 419–0018) was employed for the analysis. The TGS–2 consists consists of:

- TGS–2 Analyzer Unit
- Heater Control Unit
- Balance Control Unit
- Chart Recorder

The analyzer unit is a sensitive micro–balance that suspends the sample in a small furnace. A precisely controlled temperature profile can be programmed on the microprocessor controller. Temperatures up to 1000°C can be obtained in the furnace. During a run, the sample weight and furnace temperature are continuously displayed on the chart recorder. The absolute sample weight or the percent weight change can be recorded, as desired. The gaseous atmosphere in the sample furnace can be selected by the system operator.

The procedures followed for calibrating the system and analyzing the GAC samples are described in the equipment manuals provided by Perkin Elmer. No modifications to equipment or procedures were required.

5 BIOREACTOR STUDY

5.1 EXPERIMENTAL BIOREACTOR DESIGN

The design, construction and operation of a continuous–flow recyle bioreactor (CFRB) accounted for the greatest portion of the experimental effort of this research program. The objective of the experimental design was to concentrate the active biomass on particles of granular activated carbon rather than as suspended cells. In fact, it was desired that the concentration of suspended biomass be kept to a minimum so that the biological activity of the reactor system would be largely a result of immobiiized biomass activity. Figure 5.1 is a schematic of the CFRB design. The reactor consisted of a well–mixed tank to which the model wastewater was continuously fed. Liquid holdup in the tank was maintained constant, at approximately 460 mL, by an effluent overflow. The most important feature was a recirculation loop which incorporated a 3.2 cm I.D. column containing a bed of activated carbon. Medium was continuously pumped from the tank, through the GAC bed, and back to the tank.

The following operating criteria were critical to the design of the CFRB experiments:

1. The rate of medium recirculation was maintained very high relative to the the reactor feed rate and the phenol degradation rate of the immobilized culture. This was to ensure that the percent conversion of phenol per pass in the column was small and, therefore, the system could be considered to be well–mixed. This assumption simplifies the kinetic analysis.
2. The overall dilution rate used was several times greater than the specific growth rate of a suspended methanogenic culture to ensure the wash–out of suspended cells.
3. A steady–state with respect to the phenol concentration within the reactor

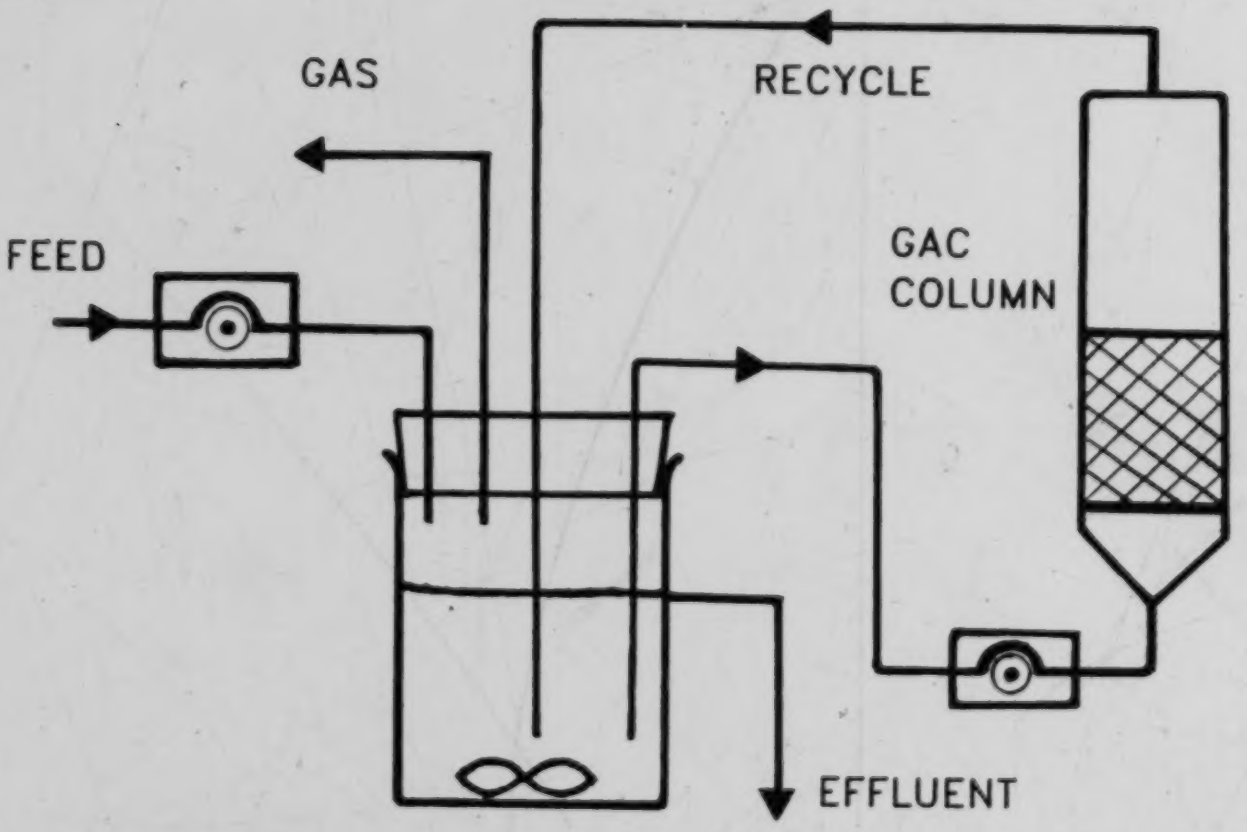


FIGURE 5.1: Schematic of Experimental Reactor (Feed System, Gas and Effluent Collection Systems not Shown)

was achieved so that the net adsorption and desorption of phenol was zero. Adsorption of phenol onto GAC is an equilibrium process which can be described by an adsorption isotherm. It was, therefore, also important that the phenol concentration was measurable to ensure that an adsorption equilibrium did in fact exist (*i.e.* that breakthrough of phenol had occurred). Doran and Bailey (1986) used a similar experimental bioreactor design to study the fermentation properties of the yeast *Saccharomyces cerevisiae* immobilized on gelatin.

The source of inoculum for the bioreactor was a 2–L phenol–degrading enrichment culture. This culture had originally been inoculated with anaerobic sludge from a domestic sewage treatment operation and had been maintained on a synthetic feed containing phenol as the major carbon source for a period of over three years. The same synthetic feed was employed as the model wastewater in the bioreactor. System temperature was maintained at the same temperature as the inoculum source *i.e.* 35°C. The support medium selected was F300 granular active carbon, manufactured by Calgon Corporation, sieved to 20 – 40 U.S. standard mesh size. Kindzierski (1989) employed the same GAC support and medium in his experiments, therefore, some basic knowledge of the performance of this material as a biological support in the anaerobic phenol–degrading system already existed.

Anaerobic conditions were achieved inside the reactor by ensuring that the medium employed was prepared and reduced as described in Section 4.2., that the oxygen was purged from the system prior to start–up and that the entire system was sealed from the atmosphere during operation. Materials were selected and procedures designed carefully so that oxygen transfer into the system would be minimized. Other features of the system were:

– transparent glass construction to permit qualitative observation of the system,

– feed system with variable speed peristaltic pump to allow variation of dilution rate,

– reversible, variable speed, peristaltic pump for medium recirculation to allow various modes of GAC bed operation (*i.e.* packed, expanded, fluidized),

– effluent collection and measurement system,

– liquid displacement gas collection system,

– reactor liquid and headspace gas sampling systems,

– total liquid hold–up of approximately 600 mL,

– GAC hold–up of 5.1 g.

A detailed description of the entire reactor system is provided in Appendix I.

5.2 PSEUDO STEADY–STATE KINETIC ANALYSIS

The amount of wash–out resistant flocculated biomass which had accumulated in the bioreactor was observed to be slowly increasing with time as a consequence of natural biological growth. As a result, a true steady–state, in which the biomass content of the reactor would be constant, could not be achieved. A pseudo steady–state assumption was made which exploited the slow growth of the anaerobic culture. If the phenol loading and degradation rates are sufficiently large relative to the biological growth rate, then the concentration of phenol in the reactor will change very slowly. Over short time periods, the change in biomass and phenol concentration would be small. A pseudo steady–state would prevail from which a reasonable estimate of phenol–degrading activity could be obtained. A second important assumption concerning biomass growth is that, after a sufficient duration of operation, the GAC would become fully–loaded with attached biomass and any further growth on the GAC would be balanced by removal mechanisms. This condition was observed by Kindzierski (1989) in serum bottle cultures after 75 d of growth. Negligible change in the quantity of flocculated biomass and

constant biomass concentration on the GAC were the major assumptions made in the kinetic analysis.

In a completely mixed, continuous system, at steady–state with respect to substrate concentration, the mass balance on substrate is:

$$V_o = F\,(C_o - C) \tag{5.1}$$

where:

F is volumetric flowrate,

C_o is feed substrate concentration,

C is reactor substrate concentration,

V_o is the overall biodegrading activity (rate) of the reactor.

In a system containing GAC and an adsorbable substrate, the amount of adsorbed substrate is also constant at constant concentration in the medium, therefore, the term for substrate adsorption is not required in the steady–state equation.

Three forms of biomass were considered to exist in the reactor; the attached, flocculated and suspended forms. The reactor biodegrading activity (or rate) is the sum of the corresponding contributions from each of these forms as described by the following equation:

$$V_o = V_c + V_f + V_s \tag{5.2}$$

where:

V_c is the biodegrading activity of the attached culture,

V_f is the biodegrading activity of the flocculated culture,

V_s is the biodegrading activity of the suspended culture.

The specific biological activity, or degradation rate, is defined here as the phenol–degrading activity per unit of biomass. If all forms of biomass in the system

are assumed to exhibit the same specific activity, then the overall activity of the reactor can be represented by:

$$V_o = \nabla_o \cdot m_b \tag{5.3}$$

where:

∇_o is the overall specific biodegrading activity of the reactor,

m_b is the amount of biomass in the system.

In this study the specific activities of the attached, flocculated and suspended biomass were not assumed to be the same. Expressions for the individual activities of these biomass forms, in terms of specific activities are:

$$V_c = \nabla_c \cdot m_c \cdot x_{b/c} \tag{5.4}$$

$$V_f = \nabla_f \cdot m_f \tag{5.5}$$

$$V_s = \nabla_s \cdot X \cdot V_l \tag{5.6}$$

where:

∇_c is the specific degradation rate of the GAC attached culture,

∇_f is the specific degradation rate of the flocculated biomass,

∇_s is the specific degradation rate of the suspended biomass,

m_c is the mass of GAC,

$x_{b/c}$ is the mass concentration of biomass on the carbon,

X is the concentration of suspended biomass,

V_l is the liquid volume of the system,

m_f is the mass of flocculated biomass.

The overall biological degradation rate, V_o, is easily determined from pseudo steady–state data using equation 5.1. Further information is required to determine the activities of the individual culture forms. At very high dilution rates, the contribution of suspended cells will be negligible because they are essentially washed

out of the system. A novel technique of separating the flocculated biomass from the GAC particles inside the reactor while maintaining an active anaerobic culture was developed. This technique, which will be discussed in more detail in a later section, involved a hydraulic separation based on the difference in density between the two materials. Flocculated biomass was completely removed from the column and collected in the tank portion of the reactor while the GAC remained in the column.

The tank contained no GAC and the biodegrading activity of the flocculated biomass, V_f, was determined in a batch test with the GAC column isolated. The difference between this batch activity and the overall reactor activity, V_o, determined from the pseudo steady–state data yielded the activity of the attached culture, V_c, as described by this equation:

$$V_c = V_o - V_f \qquad (5.7)$$

V_s was assumed to be zero because the concentration of suspended biomass is very low.

Determination of the specific phenol–degrading activities of the attached and flocculated cultures required measurement of the parameters m_c, $x_{b/c}$, and m_f in equations 5.4 and 5.5. Ideally, a quantitative measure of active biomass would be used. This measurement would include live, phenol–degrading cells and would not include dead or inactive cells and extracellular organic material that is associated with the cells. Unfortunately, such a measure is not available for mixed–population systems, including anaerobic systems anaerobic systems, principally because of the complexity of the consortia involved. The quantification of biomass used in this study was the volatile suspended solids measurement or VSS, described in Section 4.8. It measures the quantity of volatile organic material in a known volume of liquid by a simple gravimetric technique. Measurement of the quantity of

flocculated biomass, m_f, was by direct application of the VSS method. Measurement of $x_{b/c}$, the mass of attached biomass per mass of GAC support, was less straightforward and required a modification of the VSS technique using thermogravimetric analysis equipment (see Section 4.9). The activity of the suspended biomass was determined by performing a batch–degradation experiment on the reactor inoculum.

The above method allows determination of the biological degradation rates at a single phenol concentration. The specific substrate degradation rate, however, is generally a function of the substrate concentration in most biological systems. Neufeld *et al.* (1980) applied a substrate inhibition equation of the following form to describe anaerobic phenol biokinetics:

$$V = \frac{V_{max}}{\left[1 + (K_s/S) + (S/K_i)^n \right]} \qquad (5.8)$$

where:

- V is the substrate biodegradation rate,
- V_{max} is the maximum substrate biodegradation rate,
- S is the substrate concentration,
- K_s is the Monod kinetic half–rate,
- K_i is the inhibition constant,
- n is order of inhibition.

Complete evaluation of the equation parameters V_{max}, K_s, K_i and n requires determination of the substrate utilization rate at several different substrate concentrations. The above equation predicts that a substrate concentration range may exist within which the effects of substrate limitation and substrate inhibition are both very small. This occurs when S is much greater than K_s and much smaller

than K_i simultaneously. This condition does not necessarily exist for a given biological system. If it does exist the kinetic equation simplifies to a zero–order form:

$$V = V_{max} \qquad (5.9)$$

Kindzierski (1989) observed zero–order kinetic behaviour at phenol concentrations between 500 and 50 mg/L. The main purpose of the kinetic study undertaken for this research was to compare the kinetic performance of the three culture forms in the reactor. The most convenient way of doing so was to determine the maximum degradation rates by maintaining the phenol concentration within the zero–order range. In principle, a single psuedo steady–state run was required to measure V_o, V_f and V_c.

5.3 PROCEDURES

5.3.1 BIOREACTOR START–UP

The tank lid was secured in place and the feed, effluent, recirculation, gas and sample lines were attached. Filtrasorb 300 activated carbon that had been crushed, washed , and sieved to 20 – 40 U.S. standard mesh size (420 μm to 840 μm particle size) was obtained. Prior to use, the activated carbon was washed several times with dd H_2O, to remove fines, and then dried in a 105°C air oven for 48 h. 10.1 g of of dry activated carbon were added to the reactor column and the column was sealed. The butyl rubber line from the top of the column was connected to the tank but the recirculation pump tubing was left disconnected at the pump inlet. The entire reactor, including the column and all the lines, was flushed with O_2–free 30% CO_2 in N_2 for 16 h. Approximately 700 mL of boiled and gassed medium,

prepared as described in Section 4.2, were added to the reactor through the lower end of the column. As the column and the tank of the reactor were filling with medium, the tank headspace was continuously flushed with the O_2–free gas. After filling, the recirculation loop was completed by connecting the free end of the pump tubing to the butyl rubber recirculation line and the reactor was sealed by clamping the ends of the feed, effluent gas and sample lines.

The reactor was secured on the stirring plate and the column secured to a clamp stand in a 35°C walk–in incubator. The recirculation pump tubing was attached to the recirculation pump head and medium circulation and stirring were commenced. The reactor contents were reduced by adding 7 mL of 2.5% Na_2S solution. A volume of concentrated phenol stock solution was added to bring the aqueous concentration phenol to 250 mg/L after adsorption on the GAC was complete. Medium was circulated through the column for 4 d prior to inoculation on the fourth day.

Before inoculating the tank, 5 mL of a reduced 0.05 g/L resazurin solution were added to the tank to determine if the medium was reduced. (Resazurin indicator in the initial medium charged to the reactor would have been adsorbed onto the GAC during circulation.) A colour change to pink indicated that the medium was oxidized. Addition of 3 mL of 2.5% Na_2S solution was sufficient to reduce the medium in the tank. 100 mL of inoculum from the 2–L digester were transferred directly to the tank via the sample line. The transfer was made in two 50–mL stages using degassed 60 mL plastic syringes.

The gas collection system was flushed with O_2–free 30% CO_2 in N_2 and connected to the gas line from the reactor. Gas production and methane content of the headspace were monitored to ensure that a healthy, methanogenic culture was established. The culture in the tank was maintained on batch feedings of reduced concentrated phenol stock solution for a period of 8 d. On the eighth day,

circulation through the column in upflow mode was commenced. Batch feedings and monitoring of methane production continued. Occasionally, the circulation of medium was stopped and the column contents were thoroughly mixed by inverting the column several times. This was done to alleviate bed plugging problems and to ensure that all the GAC particles were adequately exposed to bacteria.

5.3.2 TRIAL PERIOD

During a trial period of 286 d duration, various modifications to the reactor system and operating procedures were made. The reactor was operated in both batch and continuous modes during this period. Phenol was available to the culture at most times, so biomass growth was continuous, and methane production continued, although at times the measured production was erratic. Problems overcome in the final design included bed plugging and channeling, plugging of the feed and recirculation lines and associated fittings, and leakage of gas from the system. The final design is as described in Appendix I.

After approximately 2 months of operation, the flocculated biomass had established itself in the column of the reactor. Figure 5.2 is a sketch indicating the locations where this biomass had concentrated. The most significant accumulation was in a layer above the GAC bed. The settled height of this top layer was approximately 1 cm. An accurate measurement was not possible because it was difficult to distinguish the boundary between the biomass layer and the GAC bed. A smaller amount of flocculated biomass had accumulated at the entrance to the column, beneath the support screen. The observation of small particles of biomass, less then a mm in size, was an indication that granules were beginning to form.

A wash–out procedure was performed 168 d following inoculation in which the flocculated biomass was removed from the column by hydraulic means. This wash–out procedure involved first washing the biomass in the bottom of the column

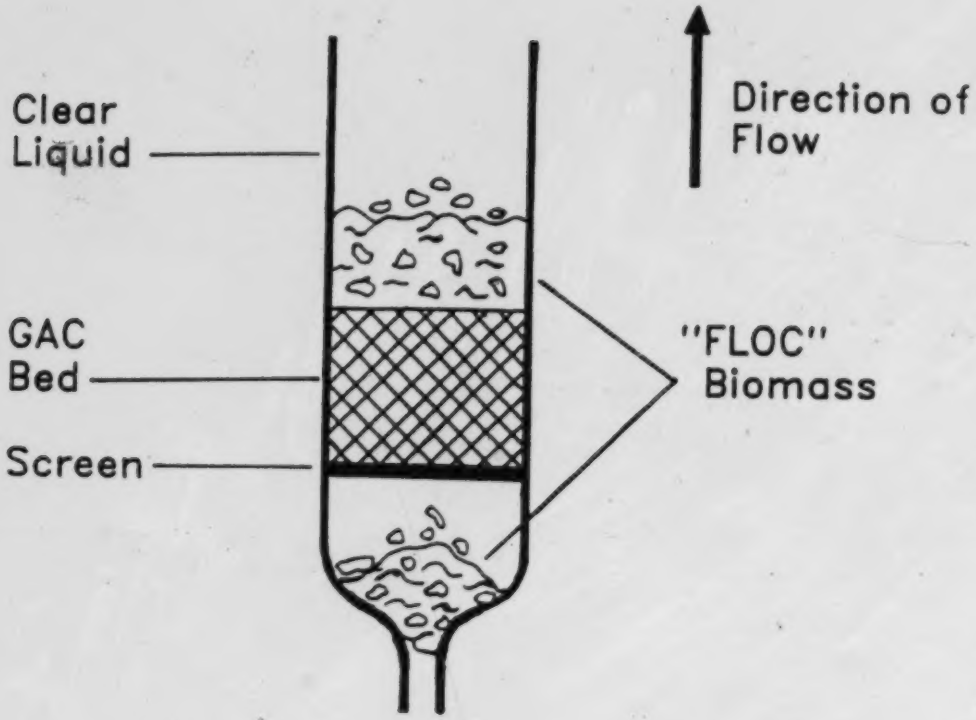


FIGURE 5.2: Locations of Flocculated Biomass in Bioreactor Column

into the tank by reversing the recirculation flow. It was necessary to turn the entire column on its side so that the bed would not plug from compression of biomass and GAC particles. The biomass in the tank was allowed to settle without stirring. After complete settling, the column was restored to a vertical position. The flocculated biomass above the GAC bed was then washed into the tank from the top of the column by increasing the upward flowrate until the GAC bed was fully fluidized (recirculation pump setting of 8). At this upward flowrate the flocculated particles were quickly washed–out of the column. The bed was left in fluidized mode for 15 min. Although the GAC bed expanded significantly during fluidization, their was sufficient freeboard above the bed to prevent entrainment of GAC particles. The biomass in the tank was again allowed to settle and the entire procedure was repeated until no flocculated biomass was observed to remain in the column. This wash–out procedure, which took about 4 h to complete, was also used at the end of the the CFRB runs.

After wash–out of the flocculated biomass, the column was isolated using screw–type pinch clamps on the butyl rubber tubing recirculation lines and separated from the rest of the reactor. The tank was dismantled and thoroughly cleaned. It was then re–assembled, gassed and filled with fresh medium using the same procedures as in the original start–up. The column, still sealed and containing the active culture attached to the GAC, was then carefully re–attached to the tank and continuous operation was commenced.

After a further two months of continuous operation the flocculated biomass was re–established at approximately the former level. This result suggests that the GAC in the reactor was saturated with biomass growth and a re–seeding of the medium was taking place. Collection of the data for the CFBR runs was commenced 286 d after inoculation.

5.3.3 CONTINUOUS OPERATION

The commencement of a CFRB run involved feed flask preparation and hook–up, preparation of the effluent collection system, zeroing of the gas collection system and setting of the recirculation flowrate. The objective during a run was to minimize disturbances to the system so that a pseudo–steady state could be achieved rapidly. During the run, the feed rate, the feed concentration and the recirculation rate were not changed. It was necessary to change effluent containers and to re–level the gas collection system.

3.5 L of medium were prepared in a 4–L Erlenmeyer flask as described in Section 4.2. After bubbling with O_2–free 30% $C0_2$ in N_2 until the pH was between 6.9 and 7.1, a pre–weighed quantity of reagent grade phenol was added to the medium. Bubbling continued for an additional 10 min and the pH was checked to ensure that it was still within the desired range. The butyl rubber feed line, with a new piece of pump tubing attached, was flushed with O_2–free 30% CO_2 in N_2 for 10 min. The rubber stopper, to which the feed line was attached, was inserted into the flask and the secured in place with cable ties. The end of the pump tubing and the outer section of Tygon tubing at the top of the flask were clamped. 35 mL of a 2.5% Na_2S solution were added through the rubber septum at the top of the flask. The inner section of Tygon tubing was then clamped. The sealed flask was inverted several times to thoroughly mix the contents. The addition of the sodium sulphide solution resulted in the instantaneous formation of a very fine, black precipitate of iron sulphide. Before hook–up of the feed, the iron sulphide precipitate was allowed to settle to the bottom of the flask for 5 d. Otherwise, the feed pump fittings tended to become clogged with iron sulphide solids during the run.

After 5 d, the feed line was filled with feed medium by removing the clamp from the pump tubing and pressurizing the flask by connecting the O_2–free gas line to the Tygon tubing. A small amount of medium was allowed to drain from the

feed line before re–clamping. A portion of this liquid was retained for determination of feed phenol concentration by GC analysis. The feed flask was then connected to the reactor at the feed pump outlet fitting and allowed to equilibrate to the incubator temperature for 1 d before feeding was commenced.

The effluent collection cylinder was flushed with O_2–free 30% CO_2 in N_2 for 30 min. A solution composed of 100 mL dd H_2O, 5 mL of resazurin and 30 g of NaCl that had been boiled for 2 min was added to the cylinder while flushing continued. One millilitre of 2.5% Na_2S solution was then added to reduce this solution. The cylinder was then sealed with a rubber stopper and transported to the incubator where it was rapidly connected to the rest of the effluent collection system. The initial liquid level in the cylinder was measured. The purpose of the salt and resazurin solution was to stop biological activity and gas production and to provide an indication of whether the effluent was reduced. (Resazurin indicator in the reactor feed was adsorbed onto the GAC and therefore did not provide any indication of the oxidation state of the reactor contents).

Before commencement of the run, the level in the gas collection system was raised to near the top of the collection cylinder by the method described in Appendix I. The recirculation pump was set at 1.0. This setting provided an upward flowrate through the column of 45 mL/min and a superficial velocity of 7.5 cm/min. This resulted in a GAC bed expansion of approximately 12% and was sufficient to alleviate problems of bed plugging and channeling. A significant amount of the GAC originally added to the column was lost during the start–up and trial periods. The height of the settled GAC bed in the column at the start of the CFRB run was 19 mm. The original height, before inoculation, was 37 mm. The mass of GAC remaining in the column was, therefore, estimated to be 5.1 g.

A feed pump setting of 0.8 was employed during the CFRB runs. A nominal feed flowrate of 350 mL/d was supplied by the pump at this setting. The ratio of

the recirculation rate to the feed rate was 188 to 1 and the system was, therefore, assumed to be well–mixed. Based on a total liquid hold–up of 608 mL, the hydraulic residence time of the reactor was 1.8 d. A single feed flask was capable of delivering over 5 times the liquid hold–up of the column. This was deemed sufficient to ensure that the reactor had adjusted to the feed concentration by the time the contents of the feed flask were depleted. The transient disturbance in reactor concentration in an ideal continuous stirred–tank reactor (CSTR) can be shown to be greater then 99% complete at 5 residence times following a step change in the feed concentration (see Appendix II.E).

The following operations were performed daily during the course of the CFRB runs:

1. A 1–mL liquid sample was withdrawm from the tank (See Appendix I for method) for determination of phenol and acetic acid concentrations by GC analyses by the methods of Sections 4.4 and 4.5.
2. The volume of gas in the gas collection system was measured and the cumulative mmoles of dry gas produced was calculated.
3. The liquid level in the effluent container was measured and the feed flowrate was checked.
4. To replace the volume of medium removed by continuous feeding, 350 mL of O_2–free 30% CO_2 in N_2 were added to the headspace of the feed flask, through the rubber septum at the top of the flask, using several 60–mL pre–flushed plastic syringes fitted with 22 gauge needles.

The samples withdrawn from the reactor were either analyzed immediately or stored in sealed test tubes at $-4^{\circ}C$ until analysis. The daily withdrawal of 1 mL was considered negligible compared to a feed flowrate of 350 mL/d. An average feed flowrate for the run was determined by dividing the total volume collected by the duration of the run. When the effluent container reached capacity it was replaced

with an identical unit prepared in the same manner as the first and a VSS determination was done on the effluent collected using the method of Section 4.8. It was necessary to filter 250 mL of effluent for each determination because of the very low biomass concentration. The headspace of the tank was sampled on the final day of the run for determination of methane concentration. Five 0.1 mL gas samples were withdrawn for GC analysis as described in Section 4.6. The run was considered complete when the feed flask was almost empty.

As soon as the run was completed the flocculated biomass was washed–out from the column, as previously described in Section 5.3.2. After collecting the flocculated biomass in the tank, the column was isolated by clamping the recirculation lines. Mixing of the tank contents was commenced. The concentration of phenol in the tank was raised to between 200 and 300 mg/L by addition of a small volume of concentrated phenol stock solution. The batch degradation of phenol in the tank was monitored over a 8 to 10 h period. Samples (0.5–mL) were withdrawn from the tank every 1.5 to 3 h and the phenol concentration of the samples was determined by GC. At the end of the sampling period, a 20 mL sample was withdrawn for determination of the VSS concentration of the tank. Combined with a knowledge of the tank volume, the VSS measurement gave an estimate of the quantity of flocculated biomass that was washed–out from the column. The flocculated biomass was washed back into the top of the column in preparation for the next run.

5.3.4 DEGRADATION KINETICS OF BIOREACTOR INOCULUM

The 2–L digester which served as an inoculum source for the CFRB experiment had been enriched on a phenol containing feed for approximately 3.5 years prior to the start–up of the bioreactor. The digester was maintained on a draw and feed basis. Before each feeding, digester stirring was stopped to allow

suspended biomass to settle. A portion of the supernatant was withdrawn and replaced by an equal volume of feed solution composed of the synthetic medium, described in Section 4.2, and phenol. Stirring was then resumed. The digester was fed 3 times per week. The liquid phenol concentration was approximately 250 mg/L after each feeding and decreased to below detectable levels within 24 h.

The specific degradation of the inoculum was determined by monitoring the rate of phenol degradation in the 2–L digester after a regularly scheduled feeding. Liquid samples (0.5 mL) were withdrawn for determination of phenol concentration by GC every 2 h using a sampling procedure similar to that employed with the CFRB. Stirring in the digester was maintained during sampling. The 2–L digester was equipped with a gas collection system to allow measurement of volumetric gas production as well. Samples were collected and gas production was measured for a period of 24 h after which time a 20 mL sample was withdrawn for determination of the VSS concentration of the digester.

5.4 RESULTS

5.4.1 CFRB RUNS

Data and results of two successful CFRB runs are presented in this section. Detailed calculations involved in the development of the results are presented in Appendix II. A complete CFRB run consisted of two distinct phases; the continuous (pseudo steady–state) phase and the batch degradation phase. The two runs were carried our consecutively using the same reactor and cultures established within the reactor. The first run, Run 1, was commenced 286 d after the initial inoculation and both phases were completed within 11 days. Run 2 was initiated 10 d after the conclusion of Run 1 and was similarly completed within 11 d. During the 10–d period between runs, the reactor was maintained in batch mode.

The important variables measured during the continuous phase are presented in Table 5.1 and Figures 5.3 and 5.4. Phenol loading rate was the major adjustable parameter in the system. The loading rate could be varied by manipulating the feed phenol concentration and the volumetric flowrate of the feed. It was more convenient to maintain a fixed flowrate and to vary the feed concentration. The phenol loading rates selected resulted in reactor phenol concentrations that were easily measured by the GC method described in Section 4.4 and were within a range in which the kinetics of substrate degradation were known to be zero–order (*i.e.* 50 – 500 mg/L, Kindzierski, 1989).

The concentration of biomass, measured as VSS, in the effluent was low in both runs (28 mg/L and 14 mg/L in Runs 1 and 2, respectively). Biomass content in the effluent was largely a result of the occasional wash–out of biomass flocs from above the GAC bed and the shearing of bacterial growth, that had accumulated on the outer walls of the glass tubing in the tank, rather then genuine suspended growth. The supernatant liquid in the reactor was observed to be clear during the CFRB runs. Two important points were suggested from these observations. First, the high dilution rate proved effective in maintaining the concentration of suspended cells at a very low level and in providing a selection pressure for immobilized growth forms in the reactor. Second, the new biomass produced by re–seeding of the supernatant by the GAC attached culture was concentrating as flocculated biomass.

The profiles of phenol and acetic acid concentration with time during the continuous phases of Run 1 and Run 2 are shown in Figure 5.3. and 5.4, respectively. The day when feeding was commenced from a newly prepared feed flask was designated as day 0. Similar trends were observed with respect to reactor phenol and acetic acid concentrations in both runs. Phenol concentration rapidly increased from a low level, initially, to a maximum after 1 or 2 d operation and

TABLE 5.1: PSEUDO STEADY-STATE DATA FROM CONTINUOUS PHASE OF CFRB RUNS

	RUN 1	RUN 1
AVERAGE FEED FLOWRATE (mL/d)	332	343
FEED PHENOL CONC. (mg/L)	639	803
FINAL REACTOR PHENOL CONC. (mg/L)	51	208
FINAL REACTOR HAc CONC. (mg/L)	330	293
AVERAGE EFFLUENT VSS (mg/L)	28	14
FINAL % CH_4 IN PRODUCT GAS[1]	75	75

Notes:

1. on a dry gas basis

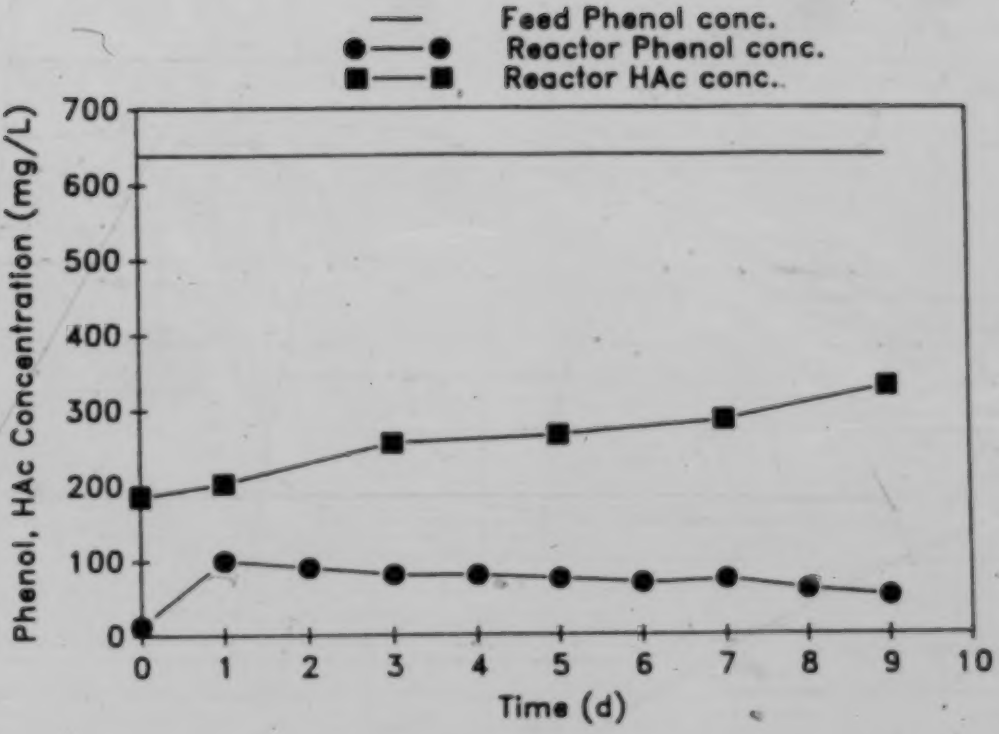


FIGURE 5.3: Pseudo steady-state phenol and acetic acid concentration profiles with time in CFRB RUN 1

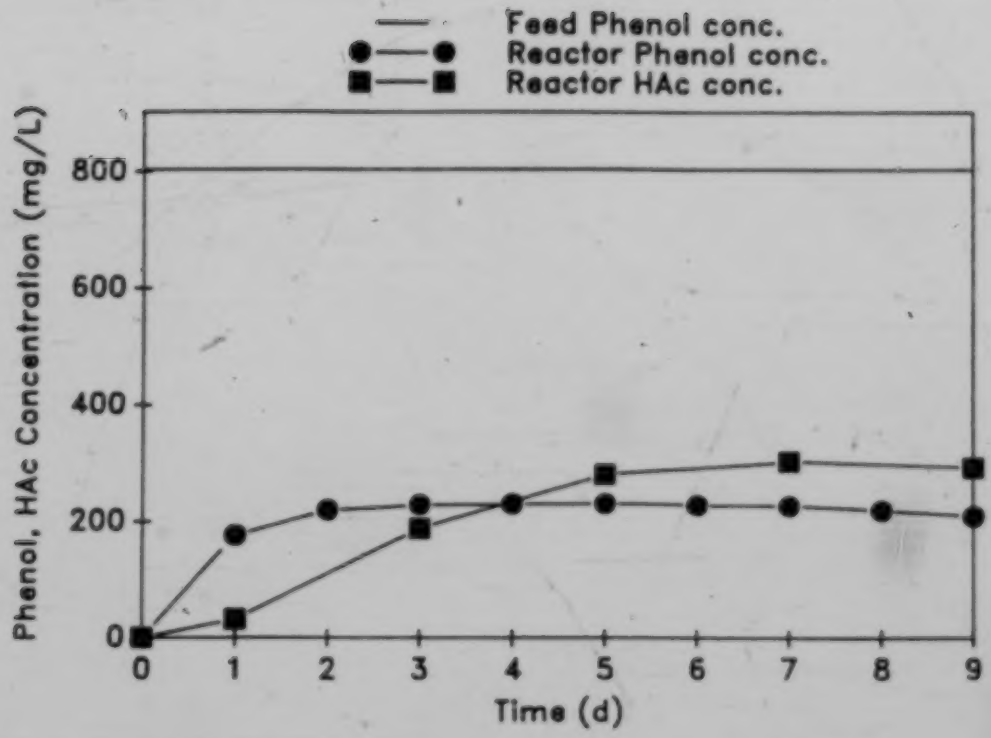


FIGURE 5.4: **Pseudo steady-state phenol and acetic acid concentration profiles with time in CFRB RUN 2**

then gradually declined until termination of the continuous phase. The initial increases were caused by positive step changes in phenol feed concentration. The gradual decline which followed the adjustment of the reactor to the higher feed concentration reflects the slow accumulation of active biomass and illustrates the difficulty of achieving a true steady–state in the reactor.

The concentration of the intermediate product of degradation, acetic acid, increased to significant levels in both runs. As Figure 5.4 shows, the acetic acid was completely degraded during the batch phase between runs but the concentration rapidly returned to previous levels after continuous feeding was commenced for the second run. Figures 5.3 and 5.4 tend to suggest that the acetic acid level was approaching a similar steady–state concentration in both runs of approximately 300 mg/L. This level of acetic acid did not appear to inhibit phenol degradation or gas production.

The accumulation of product gas in the gas collection system during the continuous phases of Run 1 and Run 2 is shown in Figure 5.5 and Figure 5.6 respectively. Continuous accumulation of gas and a high concentration of methane in the reactor headspace at the conclusion of the runs (Table 5.1) confirms that a healthy methanogenic population was established in the reactor. Linear regression fits of the data of Figures 5.5 and 5.6 yielded gas production rates of 4.3 mmole/d and 3.5 mmole/d (total dry gas) respectively. Correlation coefficients of the linear fit were 0.998 and 0.993, respectively. Because the amount of active biomass retained in the reactor was expected to be greater in the second run, due to growth, gas production in the second run was expected to be slightly greater. The gas observed gas gas production is not consistent with this expectation. The data of Figure 5.6 exhibits a discontinuity in the trend of gas production around the sixth day which tends to lower the calculated production rate. In addition, during the trial period, measured gas production was often erratic, even though phenol

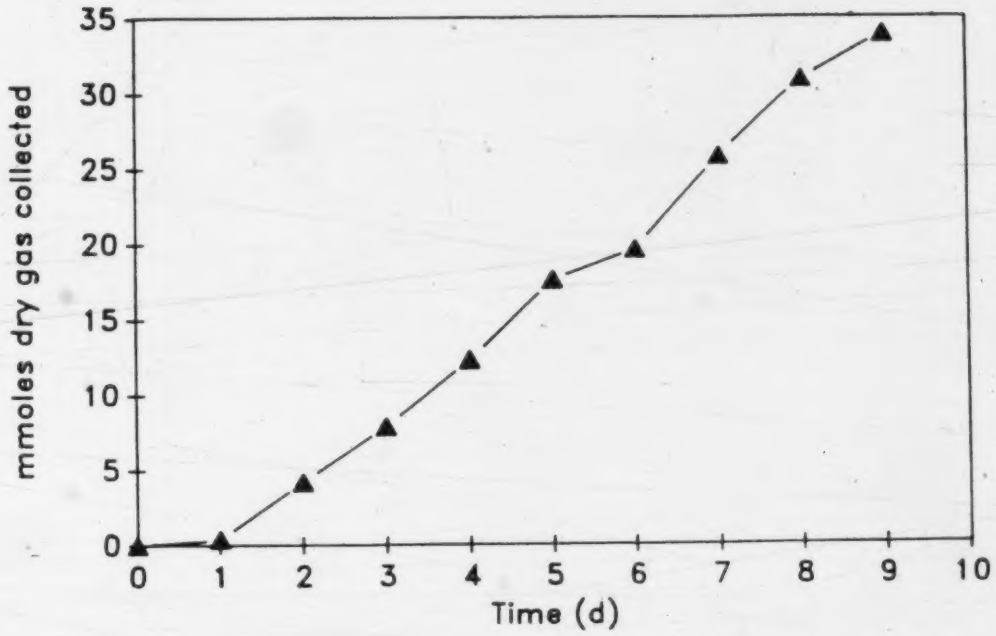


FIGURE 5.5: Measured cumulative total dry gas production during pseudo–steady state phase of CFRB RUN 1

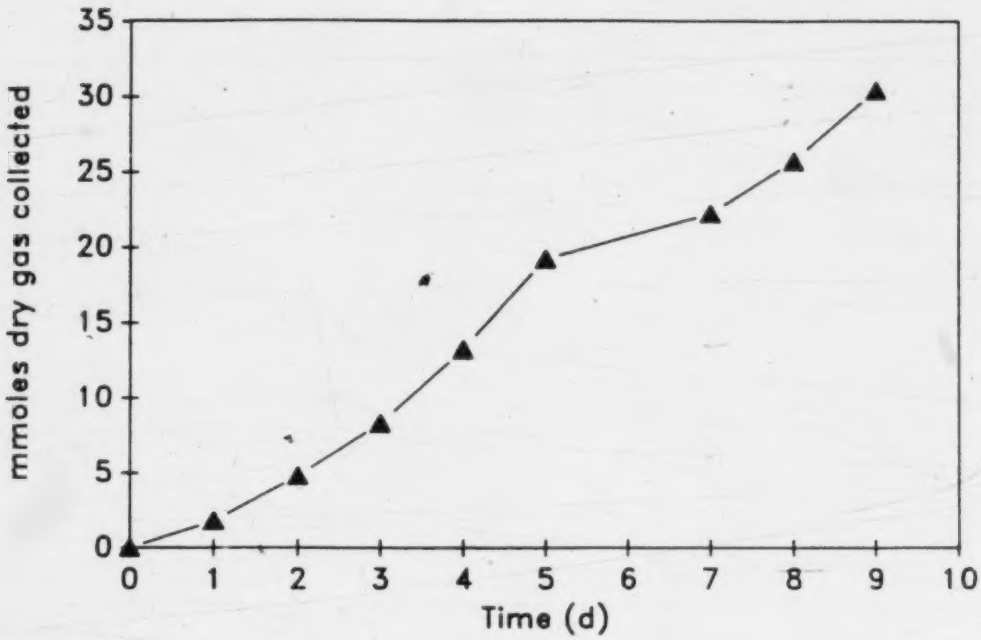


FIGURE 5.6: Measured cumulative total dry gas production during pseudo–steady state phase of CFRB RUN 2

degradation continued. These facts suggest that leaking of product gases from the system may have been occurring at one of the many joints and fittings or through the effluent collection system. Quantitative measurements of gas production in the reactor, therefore, were not considered to be very reliable.

The column wash–out procedure was carried out on the ninth day of the CFRB runs, after termination of continuous feeding. As soon as wash–out was complete, the batch experiment to determine phenol degradation activity of the tank contents was commenced. Batch degradation in Run 1 was initiated by addition of 3.5 mL of 29 400 mg/L concentrated phenol stock solution to the well–stirred tank. Two millilitres of the same solution were added to start the batch degradation of Run 2. Monitoring of the decline in phenol concentration began shortly afterwards. Linear least–squares fits of the data of Figure 5.7 and Figure 5.8 yielded phenol degradation rates of 10.1 mg/L/h and 12.0 mg/L/h, respectively. Correlation coefficients were, respectively, 0.992 and 0.9995. This indicated that the linear fits were very good and that the assumption of zero–order substrate degradation kinetics in the range of concentrations of the batch tests (*i.e.* 110 mg/L–270 mg/L) was reasonable. The absolute phenol–degrading activity of the tank contents after wash–out, V_f, was determined from the batch activity results and a knowledge of the average liquid volume in the tank. (The liquid volume varied slightly during the experiment as a result of sample withdrawals.)

A breakdown of the overall phenol–degrading activities in the reactor, showing the relative contributions of the flocculated and GAC attached biomass components is provided in Table 5.2. The overall degradation rate in the reactor, V_o, was determined by application of equation 5.1 to the pseudo–steady state data. The value of substrate concentration, C, used in equation 5.1 was the phenol concentration of the last sample taken during the continuous phase of each run (see Table 5.1). The justification for using this value in the kinetic analysis is as follows.

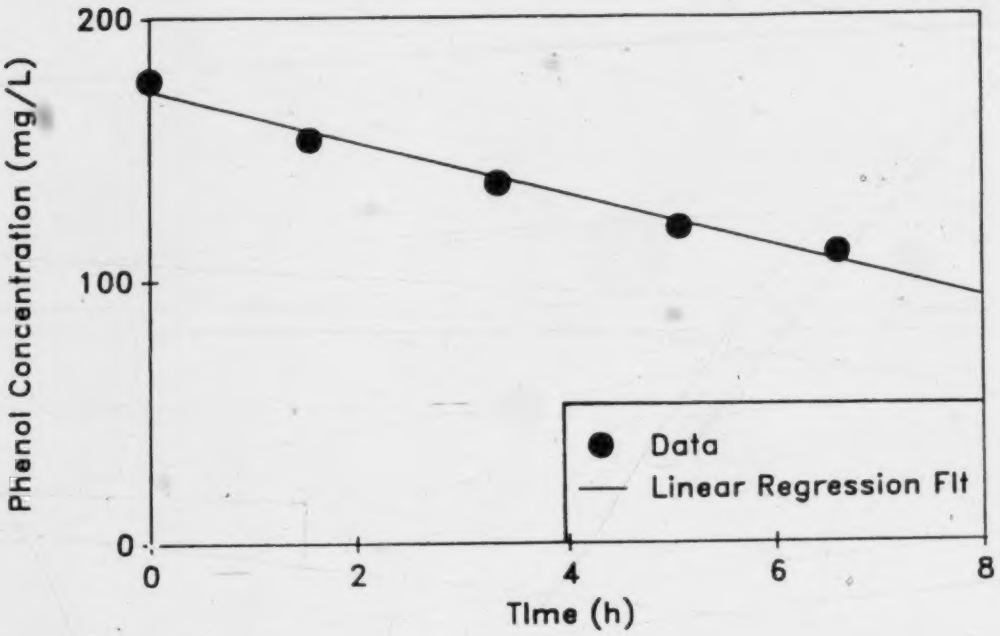


FIGURE 5.7: Phenol degradation in bioreactor tank during batch phase of CFRB RUN 1. Linear least–squares best fit is shown.

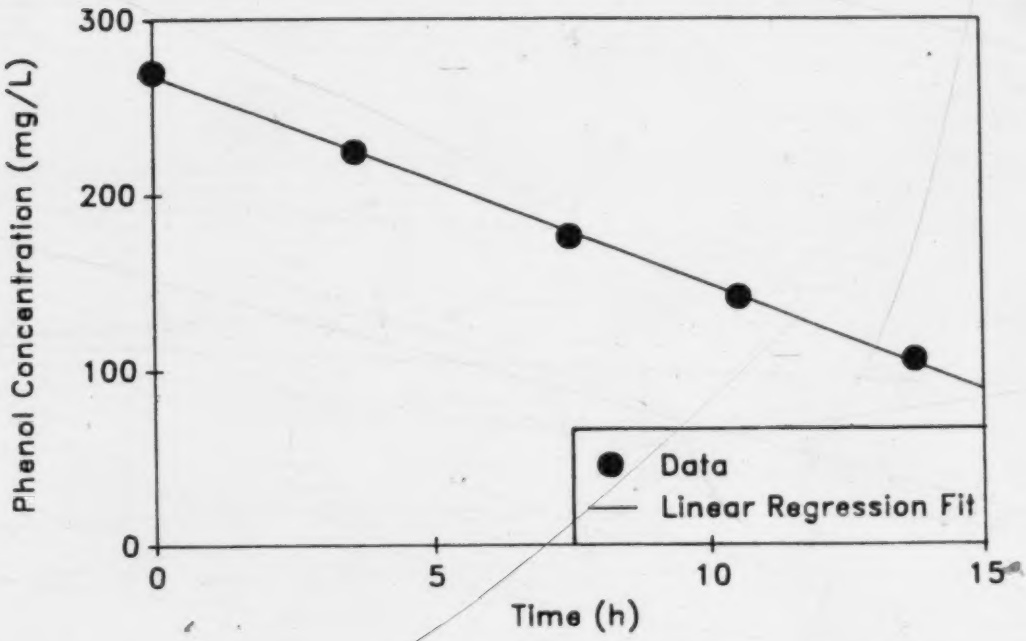


FIGURE 5.8: Phenol degradation in bioreactor tank during batch phase of CFRB RUN 2. Linear least–squares best fit is shown.

TABLE 5.2: BREAKDOWN OF PHENOL–DEGRADING ACTIVITIES IN REACTOR DURING CFBR RUNS

	RUN 1	RUN 2
	mg phenol/d	
OVERALL REACTOR (V_o)[1]	195	204
BATCH TANK DEGRADATION (V_f)[2]	113	134
GAC ATTACHED CULTURE (V_c)[3]	82	70

Notes:

1. determined from pseudo steady–state data (Table 5.1) and equation 5.1
2. determined from batch degradatation data (Figures 5.7 and 5.8)
3. $V_c = V_o - V_f$ (equation 5.7)

– see Appendix II.C for calculations

First, five hydraulic residence times had passed since the introduction of the step–change in feed concentration at the start of the run and, therefore, the system can assumed to have adjusted to this disturbance. Second, the calculated overall degradation rate was combined with the degradation rate evaluated during the batch phase to yield the activity of the culture attached to the GAC, as dictated by equation 5.7. Figure 5.3 and Figure 5.4 show that the phenol concentration was decreasing at the end of the continuous phase of the runs and would likely have continued to decrease if the runs were extended, because of the pseudo steady–state condition. The overall reactor activity was, therefore, steadily increasing during the run and this resulted in some error in determination of the attached culture activity. This error was minimized by selecting the pseudo steady–state condition that most closely reflected the activity of the reactor at the time of the batch degradation. This condition existed, of course, at the end of the continuous phase, immediately prior to the batch degradation phase.

Evaluation of the specific phenol–degrading activities of the flocculated and attached cultures using equations 5.4 and 5.5, respectively, required quantitative estimates of the amount of these biomass forms in the reactor at the conclusion of the runs. Estimates of biomass quantities are reported in Table 5.3 as mass of volatile solids (VS), rather then volatile suspended solids (VSS), to avoid misrepresentation of the form in which the biomass existed. Although measurement of the fraction of organic solids that was volatilized in air at 550°C remained the basis for quantification, the flocculated and the attached biomass did not exist in suspended forms as the term VSS implies.

The concentration of biomass on the GAC particles removed from the reactor, $x_{b/c}$, at the end of the second CFRB run material was determined to be 0.101 g VS/ g GAC by thermogravimetric analysis (TGA). A detailed discussion TGA procedure and the reliability of the measurement is given in Chapter 7. The

TABLE 5.3: BIOMASS CONTENT OF REACTOR AT END OF CFRB RUNS

TYPE OF GROWTH	RUN 1	RUN 2
	mg volatile solids (VS)	
GAC ATTACHED ($m_c x_{b/c}$)[1]	515 (47%)	515 (41%)
FLOCCULATED (m_f)[2]	475 (43%)	632 (51%)
SUSPENDED (V X)[3]	17 (2%)	9 (<1%)
WALL GROWTH[4]	90 (8%)	90 (7%)
TOTAL (m_b)	1097	1246

Notes:

1. based on TGA determination of biomass concentration on GAC after reactor shutdown (end of Run 2)
2. based on VSS determination of reactor tank contents at end of batch phase of each run and liquid volume in the tank (corrected for suspended biomass)
3. based on average VSS concentration of reactor effluent (see Table 5.1) for each run and liquid volume if reactor during continous operation
4. wall growth collected and measured by VS method after reactor shutdown (end of Run 2) and assumed constant during both runs.

amount of GAC in the column was 5.1 g (dry, biomass–free basis), therefore, the amount of biomass attached to the GAC was estimated to be 515 mg VS. As mentioned previously, this value was assumed constant during the course of the CFRB runs.

Suspended biomass was estimated from the average VSS concentration of the effluent collected during the CFRB runs (Table 5.1) and the liquid volume of the reactor during continuous operation (608 mL). Flocculated biomass was estimated from a VSS determination of a sample withdrawn from the tank at the end of the batch degradation phase. The VSS concentration was 1050 mg/L and 1370 mg/L for Run 1 and Run 2, respectively. Standard deviations of the triplicate determinations were 110 mg/L and 89 mg/L respectively. These data were corrected for the concentration of suspended biomass and combined with the liquid volume in the tank to yield the results of Table 5.3.

A drawback of most continuous bioreactors used for determination of kinetic constants is accumulation of difficult to quantify biomass growth on the walls of the vessel. Accumulation of biomass on the inside surface of the reactor tank was eliminated by periodically using the magnetic stir bar as a scraping device, however, this method was not able to remove biomass growth that had accumulated on the walls of the glass tubing inside the tank. This tube wall growth was most dense at the gas–liquid interface. An attempt at accounting for this biomass was made by carefully collecting the material removed by scraping the walls of the tubing after shut–down and dismantling of the reactor. The amount of volatile solids removed from the tubing was determined by drying the collected material at 105°C for 24 h and then by burning it in air at 550°C for 15 min and determining the weight loss. As a best available approximation, the amount of wall growth was assumed to be constant over the course of the two CFRB runs.

As the data in Table 5.3 indicate, the proportions of flocculated and attached

biomass in the reactor were similar. The flocculated biomass was, therefore, making an important contribution to the biological activity of the reactor. Suspended growth was fairly insignificant, but the proportion of wall growth, at just under 10% of the total biomass, was not negligible. The lack of true steady–state conditions is illustrated by the 33% increase in the mass of flocculated biomass observed between the end points of the two runs.

The ultimate results of the kinetic study are presented in the form of specific degradation rates (Table 5.4) which were determined by combining the information in Table 5.2 and 5.3. In this representation of the kinetic results, the contribution of wall growth to the phenol degradation in the tank was combined with the contribution of the flocculated biomass. The contribution of suspended growth was neglected. Implicit in this analysis is the assumption that the specific degradation rates of the flocculated biomass and the biomass attached to the glass tubes is similar. Given that the system is well–mixed and, therefore, both types of cultures experience similar environmental conditions, and also that the both types of biomass are biofilm (wall growth is a biofilm attached to an inert surface, biomass flocs are isolated biofilms) this assumption is not unreasonable. For the purpose of comparison, the specific phenol–degrading activity of the reactor inoculum is included in Table 5.4.

5.4.2 DEGRADATION KINETICS OF BIOREACTOR INOCULUM

The phenol–degrading activity of a suspended culture was determined and the assumption of zero–order kinetics was verified in this experiment. The well–mixed 2–L digester was considered to represent the closest approach to a truly suspended culture that was available. An ideal suspended culture, consisting individual cells in suspension, probably does not exist for the anaerobic phenol–degrading system because of the natural tendency of the bacteria to

TABLE 5.4: SPECIFIC PHENOL–DEGRADING ACTIVITIES IN CFRB RUNS

	RUN 1	RUN 2
	mg phenol/mg VS/d	
OVERALL REACTOR (∇_o)	0.18	0.16
FLOCCULANT BIOMASS (∇_f)[1]	0.19	0.18
GAC ATTACHED BIOMASS (∇_c)	0.16	0.14
INOCULUM (SUSPENDED) (∇_s)[2]	0.15	0.15

Notes:

1. includes estimate of contribution of wall growth
2. from 2–L digester (see Section 5.4.2)

associate.

The batch phenol–degrading kinetics of the 2–L digester are shown in Figure 5.9 and the corresponding gas production is shown in Figure 5.10. A non–linearity in the degradation kinetics that corresponded to a delay in the onset of gas production was observed between time zero, shortly after feeding, and the 5 h mark. Prior to the regular feeding, there had been no phenol remaining in the digester from the previous feeding for a period of about 24 h. The delay in gas production and phenol degradation was likely a result of a lag–phase experienced by the culture rather then substrate inhibition. Recall that the phenol degradation kinetics observed during the batch phase of the CFRB runs (Figure 5.7 and Figure 5.8) were not characterized by initial non–linearities at similar phenol concentrations. The cultures in the reactor tank were not subject to an absence of phenol substrate for any period of time and would, therefore, not have experienced a lag phase. At the lower end of the phenol concentration scale, the linear degradation trend was maintained even down to concentrations of 5 mg/L. Below this level, concentrations determined by the GC method of Section 4.4 were not considered reliable.

A linear least–squares fit of the data of Figure 5.9, excluding the first three data points (considered to be lag–phase), yielded a degradation rate of 10.96 mg/L/h. The correlation of the linear fit was 0.9996. The biomass concentration of the digester liquor was determined to be 1700 mg VSS/L (standard deviation, 33 mg VSS/L). The specific activity was determined by combining these results. The digester contained a certain amount of wall–attached growth which, unfortunately, could not be quantified. The value of the specific activity of the inoculum in Table 5.4, therefore, may be a slight overestimate of the true activity of a suspended culture.

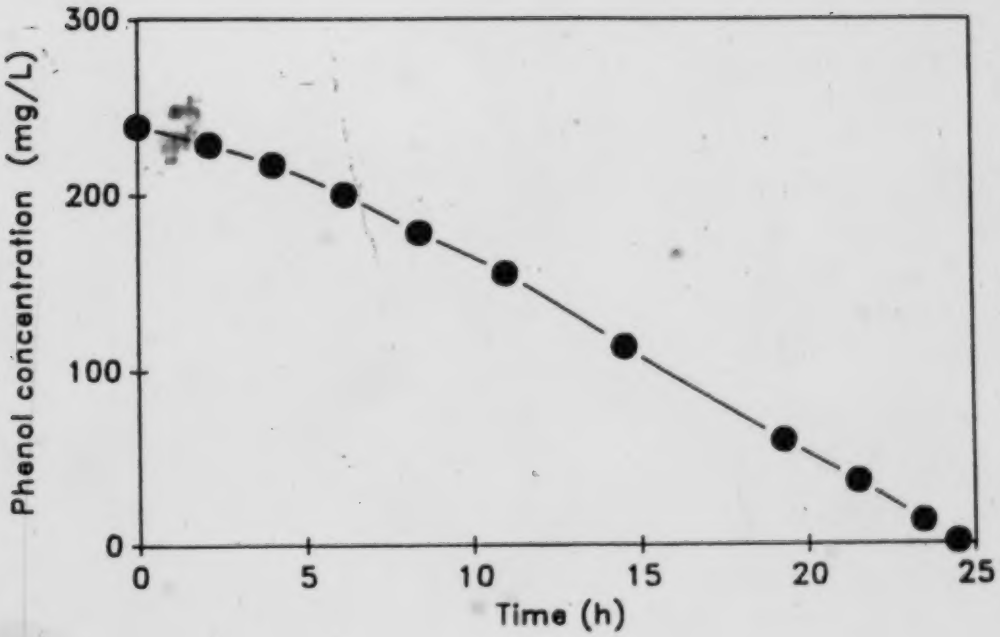


FIGURE 5.9: Batch phenol degradation in bioreactor inoculum source. i.e. 2–L continuously–stirred phenol digester, maintained for 3.5 years in draw and feed mode. Digester was fed at time 0. Phenol concentration before feeding was below detectable levels.

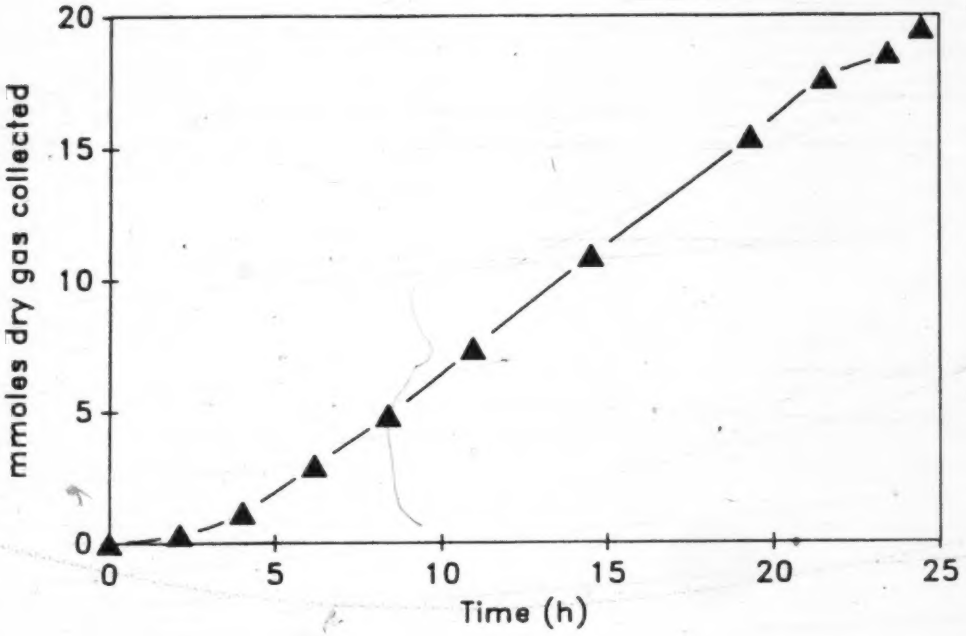


FIGURE 5.10: Measured cumulative total dry gas production in 2–L digester during batch phenol degradation experiment (Figure 5.9).

5.4.3 MASS BALANCE ON PHENOL

A mass balance was calculated for both runs in order to determine the fate of the primary substrate, phenol. The mass balances also provided estimates of the biomass yield factor but had no direct impact on the results of the kinetic analysis of Section 5.3.1. Details of the mass balance calculations are available in Appendix II.G. A summary is provided in Table 5.5.

The sinks of degraded phenol considered in the mass balance calculations were methane product, acetic acid intermediate and biomass. Phenol equivalents, the stoichiometric amount of phenol required to produce the observed quantity of the sinks, were calculated for methane and acetic acid using equations 3.1 and 3.2. Carbon dioxide product was not directly considered as a sink of phenol because it was difficult to accurately account for the amount dissolved in the reactor effluent. However, carbon dioxide production was indirectly considered by assuming the stoichiometry of equation 3.1 for the conversion of phenol to product gases. A stoichiometric relationship for the conversion of phenol to biomass was estimated from information on the elemental composition of anaerobic, phenol–degrading biomass from the 2–L digester. The biomass was washed several times with dd H_2O and then freeze dried overnight. Elemental analysis (C, H, N, O, S) was performed in the Microanalytical Lab in the Department of Chemistry at the University of Alberta, Edmonton, Alberta (See Appendix II.G).

Information on the amount of phenol in, phenol out, acetic acid and gas produced was available in the form of the daily data collected during the CFRB runs. Estimates of the biomass content in the reactor at the beginning and at the end of each run were required to complete the balance. The values given in Table 5.3 provided fairly good estimates of these quantities with the exception of the amount of biomass at the start of Run 1. This information was not directly available. Estimates were available for the biomass content in the reactor at the

TABLE 5.5: SUMMARY OF PHENOL BALANCE ON CFBR RUNS

	RUN 0/1	RUN 2
	mg phenol equivalent	
PHENOL IN	3293	2424
PHENOL OUT	285	692
PRODUCT GAS[1]	727	476
ACETIC ACID PRODUCED	632	341
BIOMASS PRODUCED	231	147
UNACCOUNTED PHENOL	1427	769
BIOMASS YIELD FACTOR[2]	0.13	0.14

Notes:

1. based on measured CH_4 production

2. units are g VS per mg phenol degraded

– see Appendix II.G for calculations

start of the trial CFRB run that preceded Run 1, however. The data for this run, named Run 0, were available and are given in Appendix II.H. A mass balance was calculated for Run 0 and Run 1 combined (designated Run 0/1).

Table 5.5 indicates that 33% and 17% of the phenol fed to the reactor over the course of Run 0/1 and Run 2 was unaccounted for in the respective mass balances. The possibility of a build–up of an unidentified metabolic intermediate, benzoate for example, was considered, however an HPLC analysis of a sample withdrawn during Run 2 showed no significant peaks other than those characteristic of phenol and acetate. This avenue was, therefore, pursued no further. A more likely explanation for the unaccounted phenol is adsorption onto the GAC. In both balances, the final phenol concentration in the reactor was significantly higher than the initial concentration, therefore, some phenol accumulated on the GAC. It was difficult to account for the quantity of adsorbed phenol because of a lack of an equilibrium relationship for the adsorption of phenol onto colonized GAC. An additional reason for the difficulty in closing the mass balance was the inability to effectively collect and measure the product gas. The lack of reliability in measurement of gas production was mentioned in Section 5.4.1. Loss of product gas through the effluent collection system was suspected despite the efforts made to prevent this. Small but measurable concentrations of methane were detected in the headspace gas of the effluent collectors during the runs.

Despite the lack of closure, the mass balances were able to provide estimates of the biomass yield factor. Biomass yield factor is defined as the mass of biomass produced, as volatile solids, per mass of phenol substrate degraded. Yield factors were estimated independently of gas production data and the agreement between values for the two runs, as Table 5.5 shows, is good.

5.5 DISCUSSION

The technique employed for the determination of kinetic parameters, the pseudo steady–state condition and the biomass separation method in particular, were novel. The reproducibility between successive runs, illustrated by the kinetic results presented in Table 5.4, supports the effectiveness of the technique. The largest difference between corresponding specific degradation rates of the tow runs was 15% (between the results for the GAC attached culture). Specific rates are normalized with respect to biomass and, therefore, account for the increase in biomass concentration in the reactor between the runs. Although the pseudo steady–state concentration of phenol was greater in the second run than in the first, the phenol–degrading kinetics were shown to be independent of concentration in the range experienced in the reactors, therefore, this should not affect the degradation kinetics. Because all other environmental conditions were identical for both runs, it is reasonable to expect similar specific degradation rates.

Recalling the initial objectives of the kinetic study, the results of Table 5.4 indicate that there was no enhancement of the kinetic ability of the phenol–degrading culture by virtue of immobilization and growth on the GAC. In fact the results suggest that the kinetic activity of the attached, flocculated and suspended forms were quite similar, with the activity of the flocculated culture being slightly greater than the other two. The specific degradation rates determined for these culture types were are all within 30% of each other. Although a slight superiority in the activity of the flocculated culture is suggested by the results, there is insufficient information to establish a strong positive correlation.

The build–up of acetic acid to the concentration observed in the reactors is worthy of some discussion. As noted earlier, the acetic acid concentration appeared to be approaching a stable value at the end of the runs. There was no accompanying decrease in gas production or phenol degradation, which would

suggest inhibition of the methanogenic or phenol–oxidizing populations. The buffering capacity of the system was therefore sufficient to prevent a decline in pH. The acetic acid build–up suggests that the phenol–oxidizing bacteria are more efficient at responding to the increased phenol loading experienced by the cultures during the CFRB runs relative to the phenol loading during the trial period. Based on the pseudo steady–state data, and assuming the stoichiometry of equation 3.2, 29% and 24% of the degraded phenol accumulated as acetic acid in Run 1 and Run 2, respectively. The slightly lower accumulation of acetic acid in the second run may be a reflection of an increase in the methanogenic population relative to the phenol–oxidizing population. Accumulation of the acetic acid confirms that methanogenesis of acetate is the rate–limiting step in the overall phenol degradation process.

The major limitations of the CFRB study are the assumptions of pseudo steady–state conditions and complete separation of the flocculated and attached biomass by the hydraulic technique. Addressing the latter point, there was no convenient method available for quantitatively verifying the efficiency of the hydraulic separation method. Verification of separation was done visually and vigorous fluidization was extended to ensure complete wash–out of the flocculated biomass. The height of the GAC bed after the wash–out procedure was identical in both runs, suggesting that the GAC was effectively retained in the column. Dismantling of the reactor in an attempt to measure the residual flocculated biomass was not considered as this would require a premature sacrificing of the active culture.

A qualitative analysis to estimate the order of the error introduced by the assumption of pseudo steady–state was possible (see Appendix II.F for details). The accuracy of calculated reactor activity was affected by the continuous increase in the reactor activity, resulting from biomass growth, and also by the desorption of

phenol from the GAC, resulting from the decline in phenol concentration. Figures 5.11 and 5.12 illustrate the steady decline in phenol concentration that occurred at the end of the continuous phase of the runs. Using data from the final three days of each run, the linearized rates of decline were 6.7 mg/L and 6.5 mg/L in Run 1 and Run 2 respectively. The similarity of these figures reflects the steady rate of accumulation of biomass in the reactor. The rates of decline tended to result in underestimation of the reactor degradation rate (see Table 5.2) by approximately 3% in both runs.

As the phenol concentration decreased, phenol was desorbed into the medium. The inability to account accurately for this desorption resulted in a further underestimation of the reactor activity. A first approximation to the magnitude of this error was obtained using the adsorption equilibrium isotherm determined by Kindzierki (1989) for adsorption of phenol on F300 GAC in growth medium at 37°C:

$$q = 44.4\ C_e^{0.17} \qquad (5.10)$$

where q is the amount mg of adsorbed phenol per g of GAC and C_e is the equilibrium concentration of phenol in the medium. Using phenol concentration data from the final three days of the continuous phase of the runs, the phenol desorption rates were estimated to be 13.5 mg/d and 5.1 mg/d for Run 1 and Run 2 respectively. The resulting under–estimations of the reactor degradation rates were about 7% and 3% . Combining the effects of the biomass increase and phenol desorption reveals that the order of magnitude of the error involved in making the pseudo steady–state assumption on estimation of the reactor activity was 10% or less.

Another assumption, implicit in the pseudo steady–state assumption, was of

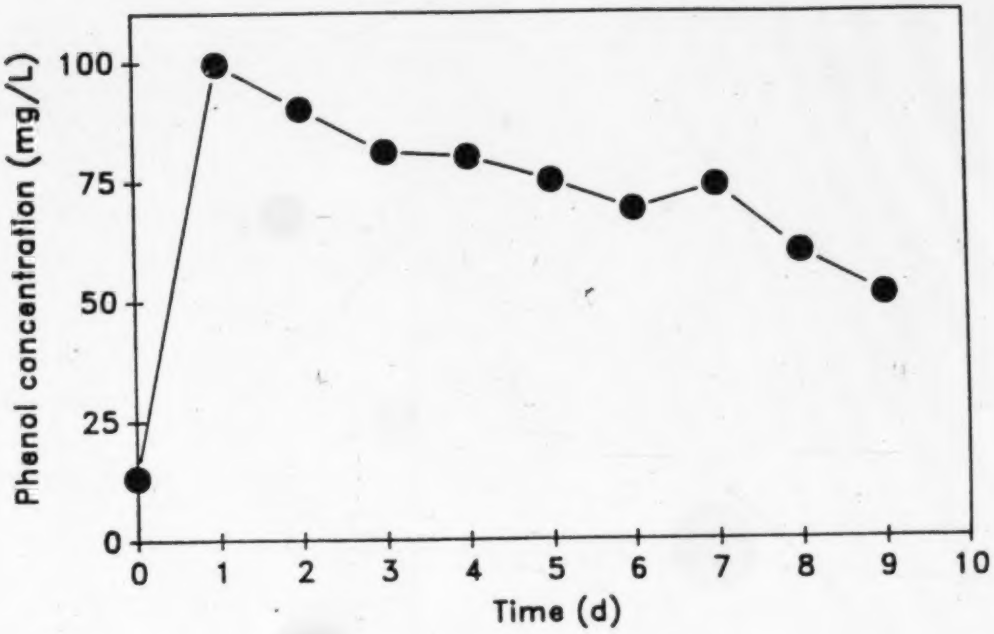


FIGURE 5.11: Phenol concentration in bioreactor during pseudo steady–state phase of CFRB RUN 1. Same reactor phenol concentration data as shown in Figure 5.3 with expanded–scale on vertical axis.

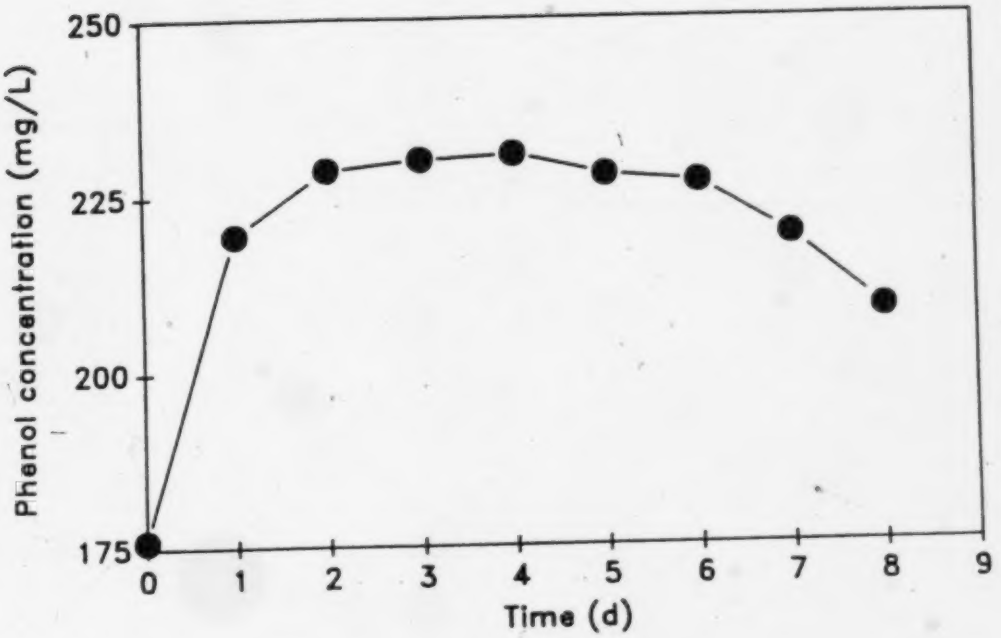


FIGURE 5.12: Phenol concentration in bioreactor during pseudo steady–state phase of CFRB RUN 2. Same reactor phenol concentration data as shown in Figure 5.4 with expanded–scale on vertical axis.

constant biomass concentration on the GAC. This could not be verified directly, however, information on the nature of the colonization, presented and discussed in Chapter 7, supports the assumption. In addition, the biomass yields determined from the mass balances (see Table 5.5) suggest that at least 7% of the phenol was being converted into biomass. This figure is typical of conversions in anaerobic systems, *e.g.* 5% reported by Sahm (1984). The mass balance assumed that all the biomass yield was concentrated in the non–attached forms. If a significant increase in attached growth occurred, the resulting biomass yield would tend to be lower then typical literature values.

Determination of specific activities relies on the estimates of biomass quantities. Estimation of the quantity of flocculated biomass was performed using the VSS technique which is of limited and variable precision. Standard deviations of triplicate determinations in Run 1 and Run 2 were 10% and 6% of the respective means. The use of thermogravimetric analysis to determine the concentration of biomass on the GAC was novel and the accuracy of the method will be discussed in Chapter 7. Wall–attached growth also presented a source of error in determining the overall reactor activity, however, the activity of the wall attached culture was accounted for in the batch degradation phase. The determination of the activity of the GAC culture was, therefore, not affected. An underestimate of the quantity of wall growth may have resulted in an over–estimate of the specific activity of the flocculated biomass.

6 SHOCK LOADING EXPERIMENTS

6.1 REVIEW OF OBJECTIVE

An important consideration in treatment process design and operation is the ability of the bacterial cultures to respond to system stresses. Of particular interest is the ability to recover from sudden surges in the concentration, or shock loads, of inhibitory substrates. Two distinct and important culture types had developed in the bioreactors during the extended period of operation. The pseudo steady–state kinetic analysis revealed that the flocculated and GAC–supported cultures were making approximately equal contributions to the overall phenol–degrading activity of the reactor under steady loading and non–inhibitory phenol concentrations. The objective of this experimental study was to determine the relative abilities of the two culture types to respond to shock loadings of phenol.

It has been established that, by virtue of adsorption, GAC serves to buffer a biological system against the effects of shock loadings of inhibitory compounds (see Section 3.2). However, It has not been shown that, apart from lowering the concentration of inhibitory compounds in the supernatant by adsorption, there exists an additional mechanism by which the bacteria are protected from the effects of shock loads. Mass transfer limitations within the colonized activated carbon particle and the attached biofilms might result in the establishment of protective concentration gradients. The bacteria, embedded in a polymer glycocalyx and supported within the surface pores and crevices of the GAC, might experience concentrations of substrate which are significantly lower than in the bulk liquid if the rate of diffusion was comparable to the rate of biological uptake. The rate of diffusion within the GAC particle is directly related to the rate of adsorption. If this mechanism was effective, it would suggest a protective feature which is related to the unique surface geometry of the GAC and the properties of the biofilm

supported within that geometry, in addition to the adsorption capacity. If the surface geometry was not a crucial factor and the rate of adsorption was much greater than the rate of biological degradation then mass transfer limitations would not be significant, and supported biofilms would have no protective advantage over unsupported biofilms, such as flocculated or granular particles.

To investigate the effect of shock loading, flocculated and GAC–supported cultures were separately subjected to nominal phenol concentrations of 1000 mg/L, 1500 mg/L and 2000 mg/L in the supernatant liquid. These concentrations were selected in order to provide varying degrees of inhibition. Recall from Section 3.1 that inhibition of phenol degradation was observed at concentrations as low as 600 mg/L, however, concentrations as high as 1400 mg/L were eventually degraded in batch cultures (Wang *et al.*, 1989). In this study, cultures of flocculated and GAC–supported biomass were established separately in serum bottles using the bioreactor as a source of inocula. Initial phenol–degrading activities, at non–inhibitory substrate levels (*i.e.* less than 300 mg/L), were determined in batch degradation tests. The cultures were then spiked with the high concentrations of phenol and the subsequent decline in concentration was monitored. Gas production was also monitored throughout the study.

Estimation of biological degradation rates from batch degradation data in GAC cultures requires information about the phenol adsorption equilibrium. This information was provided from an experimentally determined phenol adsorption isotherm. For the isotherm to be useful for this purpose, the GAC employed was the removed from the column of the bioreactor.

6.2 PROCEDURES

6.2.1 BIOREACTOR PREPARATION

The bioreactor was shutdown at the end of the batch degradation phase of the second CFRB run. At this time, the flocculated biomass had been washed into the reactor tank while the GAC remained in the column. The feed, gas and effluent lines were sealed, so that anaerobic conditions were preserved, and the tank and column unit was disconnected and removed from the incubator room in preparation for inoculation of the cultures for the shock loading experiments.

6.2.2 FLOCCULATED BIOMASS CULTURE SET–UP

Four cultures of flocculated biomass were established in 158–mL serum bottles. Three of the cultures received the shock loadings and the fourth served as a control and received no phenol. Each serum bottle was flushed with O_2– free 30% N_2 in $C0_2$ for 15 min. While flushing of the headspace continued, precisely 10 mL of non–reduced medium (prepared as described in Section 4.2) were added to each bottle. The medium was reduced by addition of 0.1 mL of 2.5% Na_2S solution. The bottles were then inoculated with 40 mL of culture from the reactor tank. The inoculum was transferred directly from the tank into the serum bottles using a 60–mL plastic syringe. The contents of the tank were well stirred at the time. A portion of the tank contents was retained for determination of the VSS concentration. After addition of the inoculum, the headspace of the serum bottles was flushed for another 5 min before the bottles were stoppered and capped. The cultures were incubated at 35°C on a platform shaker set at 200 rpm.

Each culture, with the exception of the control, received 0.35 mL of 30 640 mg/L concentrated phenol feed solution. The decline in phenol concentration was monitored for approximately 21 h and the methane concentration of the

headspace gas was checked to verify methanogenic activity. At that time, each culture received an additional 0.30 mL of 30 640 mg/L phenol feed solution and the decline in concentration was monitored as before. The pre–shock load rate of degradation was determined. After another 21 h, the shock load feedings were carried out. Culture 1 received 1.6 mL of concentrated phenol stock solution, culture 2 received 2.4 mL and culture 3 received 3.2 mL. All feedings were instantaneous. The phenol concentration in each culture was monitored over time. When the concentration dropped to less than 100 mg/L the cultures were fed another 0.3 mL of phenol feed solution and the post–shock load degradation rate was determined.

To prevent over–pressuring of the serum bottles, gas produced was periodically released from the headspace using the apparatus described by Fedorak and Hrudey (1983). The device uses an arrangement of glass syringes and two–way stop–cocks to collect and measure gas from stoppered serum bottles. The volume of gas released from the cultures during de–pressurization was measured to provide a quantative check on total gas production.

6.2.3 GAC CULTURE SET–UP

The inoculation of the GAC cultures was carried out in an anaerobic chamber (Coy Laboratory Products Inc.). The atmosphere in the chamber was 30% CO_2 and 10% H_2, in N_2. Oxygen was removed from the atmosphere by reaction with H_2 over a palladium coated, alumina catalyst. The reactor column, still full of GAC and medium, was isolated by clamping the butyl rubber recirculation lines and was disconnected from the rest of the reactor. Four 58–mL serum bottles were flushed with O_2– free 30% N_2 in $C0_2$ gas for 15 min before addition of precisely 20–mL of non–reduced medium to each. The bottles were then sealed and reduced by addition of 0.2 ml of 2.5% Na_2S solution. The reactor column and the serum

bottles were placed in the anaerobic chamber for the inoculation procedure.

After 24 h, the column and the serum bottles were opened inside the anaerobic chamber. The supernatant liquid was drained from the column and collected. Precisely 10 mL of this liquid were added to cultures 1, 3 and the control culture. Only 5 mL were added to culture 2 because of a limitation in quantity. The supernatant was observed to contain very little suspended biomass. Approximately equal portions of the wet, colonized GAC were transferred from the column into each of the serum bottles using a spatula. The bottles were then stoppered, capped and removed from the hood. As with the flocculated biomass cultures, the GAC cultures were incubated at 35°C on a platform shaker set at a speed of 200 rpm.

To obtain the pre–shock phenol degradation rates, each culture was fed 0.6 mL of 30 640 mg/L concentrated phenol feed solution. The control culture was not fed. Monitoring of the phenol concentration in the supernatant liquid was commenced several hours following feeding. Adsorption of phenol onto the GAC was assumed to have reached a pseudo–equilibrium by that time. Methane concentration in the headspace gas was periodically checked to ensure that methanogenesis was proceeding. After eight days, the shock loadings were applied. Cultures 1, 2 and 3 received 1.4 mL, 1.6 mL, and 2.2 mL of concentrated phenol stock solution, respectively. The cultures were left to degrade phenol until the concentration in the supernatant fell below 25 mg/L. At this point, the cultures were again fed to bring the supernatant phenol concentration to approximately 300 mg/L and the post–shock phenol degradation was monitored. De–pressurization and measurement of gas production was performed in the same manner as in the flocculated culture experiments.

Upon completion of the batch degradation experiments, the amount of carbon in each bottle was accurately weighed. The supernatant was removed from

the bottles, taking care to leave the GAC behind. The GAC was then washed with several volumes of dd H_2O to remove any remaining medium and biomass particles. The GAC, still in the bottles, was dried in a 105°C air oven for 48 h, cooled in a desiccator and then accurately weighed. The weight of the GAC was determined by subtracting the weight of the clean and dry serum bottles.

6.2.4 PHENOL ADSORPTION ISOTHERMS

A phenol adsorption isotherm was determined for the GAC removed from the column after reactor shutdown. The colonized GAC was dried in an air oven at 105°C for 48 h to remove moisture and previously adsorbed phenol and to terminate anaerobic biological activity. After cooling of the GAC in a desiccator, portions of the GAC (14.4 mg, 25.2 mg, 43.4 mg, 60.3 mg, 77.6 mg, 106.1 mg and 181.9 mg) were carefully weighed into pre–tared 38–mL serum bottles. Precisely 10.0 mL of non–reduced medium (pH = 7.0 ± 0.1) were added to each bottle, anaerobically. The bottles were stoppered and capped and then reduced with 0.1 mL of 2.5% Na_2S solution. After equilibrating for 24 h at 35°C on a rotary shaker (200 rpm) the supernatant liquid in the two bottles containing the largest quantities of GAC was analyzed for phenol to determine whether or not the adsorbed phenol had been removed in the drying process. No phenol peak was detected even when the sensitivity of the GC integrator was increased over the normal mode.

Stoppers were removed from the bottles and precisely 0.50 mL of a 12 476 mg/L solution of phenol in boiled dd H_2O were added to each while flushing the headspaces with 0_2– free 30% CO_2 in N_2 gas.. The bottles were stoppered and capped once more and then autoclaved at 15 psi for 20 min to ensure that all biological activity had ceased. After equilibrating at 35°C for 5 days on a rotary shaker (200 rpm), the concentration of phenol in the supernatant of each bottle was determined by GC. The amount of adsorbed phenol was calculated using the

following equation derived from a straightforward mass balance on phenol:

$$q = \frac{[V_a C_a - V_l C_e]}{m_c} \qquad (6.1)$$

where:

q is the amount of adsorbed phenol (mg adsorbed phenol/g GAC),

V_a is the volume of standard phenol solution added (0.5 mL),

C_a is the concentration of standard phenol solution added (12 476 mg/L),

V_l is the final liquid volume (10.5 mL),

C_e is the equilibrium phenol concentration,

m_c is the mass of dried GAC added to each bottle.

The adsorption isotherm was constructed from values of q versus C_e.

A second isotherm was determined for clean F300 GAC which was prepared in a manner identical to the GAC which was added to the bioreactor prior to start–up (see Section 5.3.1). The quantities of GAC used in the experiment were 20.7 mg, 36.2 mg, 51.8 mg, 75.2 mg and 100.3 mg and the concentration of the phenol solution was 12 520 mg/L. All other quantities and procedures were identical to those used in the determination of the isotherm for the GAC removed from the reactor, inluding autoclaving of the serum bottles after addtion of phenol.

6.3 RESULTS AND DISCUSSION

6.3.1 SHOCK LOADING OF FLOCCULATED BIOMASS CULTURES

The actual shock loads received by the flocculated cultures were 980 mg/L, 1430 mg/L and 1850 mg/L in cultures 1, 2 and 3 respectively. Figures 6.1, 6.2 and 6.3 show the effects of these shock loads, in terms of phenol–degrading activity.

The pre–shock load phenol–degrading activity was readily established for

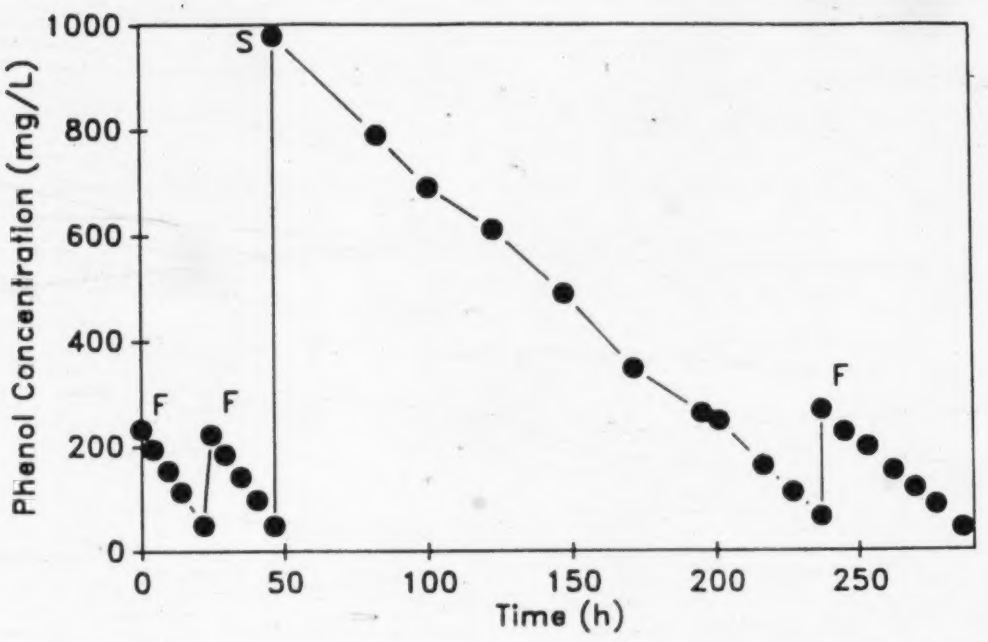


FIGURE 6.1: Response of flocculated biomass culture 1 to a phenol shock load of 980 mg/L in the supernatant. Pre–shock batch activity was determined in two sequential batch degradations at low phenol concentrations. Post–shock batch activity was verified by a final batch activity determination.

F = pulse feeding at non–inhibitory phenol level. ($ie < 300$ mg/L).

S = shock load pulse feeding to inhibitory phenol level.

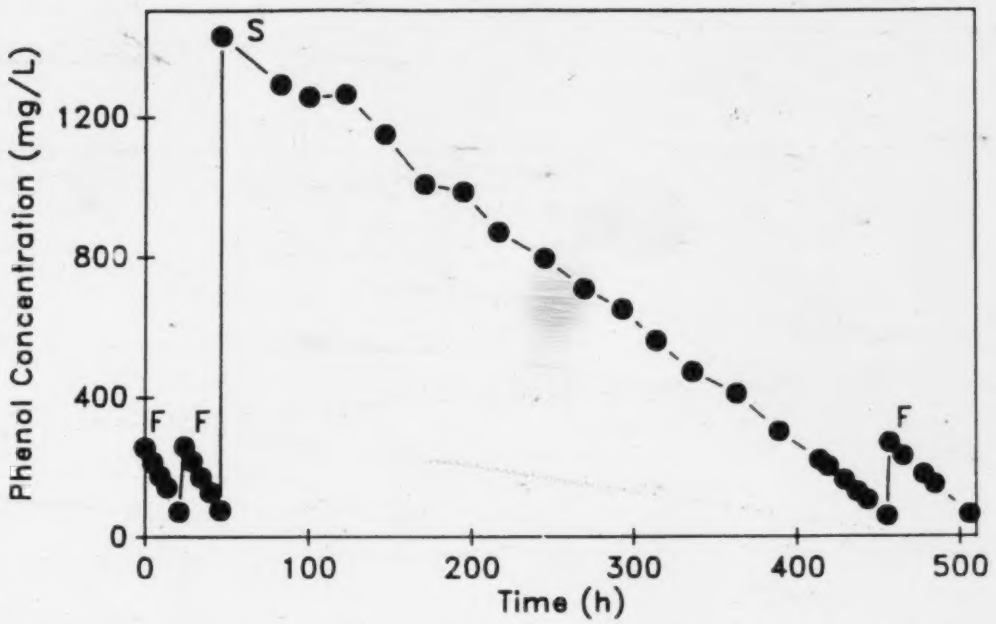


FIGURE 6.2: Response of flocculated biomass culture 2 to a phenol shock load of 1430 mg/L in the supernatant. Pre–shock batch activity was determined in two sequential batch degradations at low phenol concentrations. Post–shock batch activity was verified by a final batch activity determination.

F = pulse feeding at non–inhibitory phenol level (*i.e.* < 300 mg/L).

S = shock load pulse feeding to inhibitory phenol level.

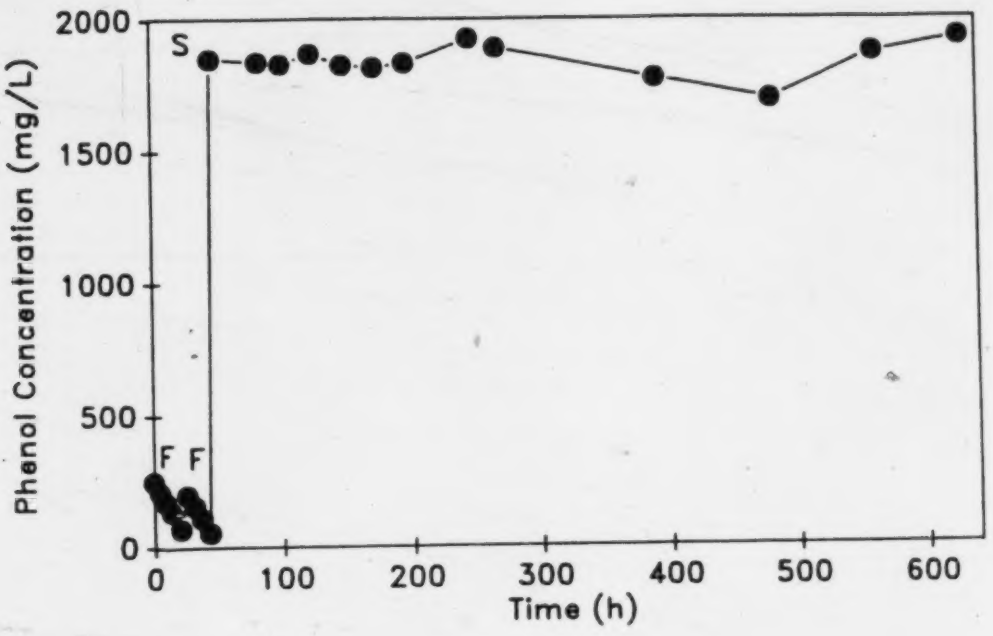


FIGURE 6.3: Response of flocculated biomass culture 3 to a phenol shock load of 1850 mg/L in the supernatant. Pre–shock batch activity was determined in two sequential batch degradations at low phenol concentrations. No measurable post–shock batch activity was observed.

F = pulse feeding at non–inhibitory phenol level (*i.e.* < 300 mg/L).

S = shock load pulse feeding to inhibitory phenol level.

each culture in two successive batch degradations. Figure 6.4 is an example of the initial batch degradation for culture 1. It is simply the initial portion of Figure 6.1 magnified for illustrative purposes. (See Appendix III for similar Figures for cultures 2 and 3.) During these initial activity determinations, the phenol concentrations were maintained below 300 mg/L to ensure that no substrate inhibition was occurring. To eliminate lag phases, the cultures were fed phenol before the previous phenol concentration had declined to less than detectable levels. Pre–shock degradation rates were estimated from slopes of linear least–squares fits of the concentration versus time data. Table 6.1 summarizes the results. The linear fits were very good. There was no significant increase in the degradation rate between the first and second batch degradations, therefore, the initial rate was taken as the the arithmetic average of the two values.

Cultures 1 and 2 responded well to the shock loadings although there was a reduction in phenol–degrading activity. Degradation of phenol commenced immediately following the shock loads. No lag phase was evident and all the phenol introduced with the shock load feeding was eventually degraded. The post–shock degradation activities were determined after the phenol concentration had declined to below 300 mg/L. A final feeding and batch degradation was performed to verify this result. Figure 6.5 is an example of the post–shock phenol–degrading activity of culture 1. Table 6.2 summarizes the results.

The phenol–degrading activity in culture 3 was rapidly and completely inhibited following a shock load of 1850 mg/L (Figure 6.3). After 25 days following the shock load, there was no recovery of activity. A least–squares analysis of the post–shock load data of Figure 6.3 yielded a slope of –0.0004 mg/L/h. The post–shock degradation rate was, therefore, considered to be zero.

Specific degradation rates, normalized for biomass content, are given in Table 6.3. The pre–shock specific rate was based on a VSS determination of a

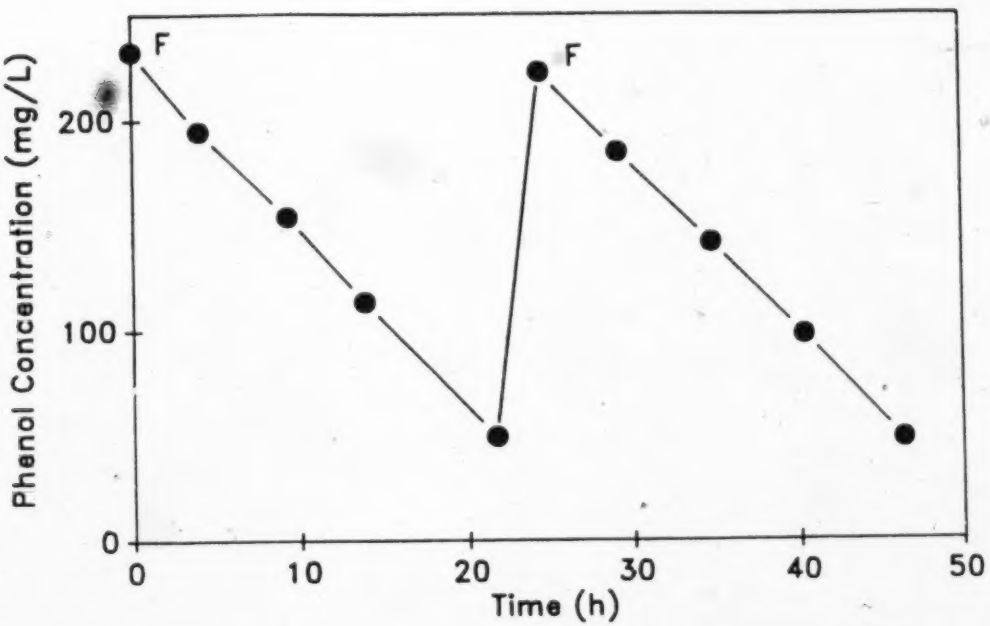


FIGURE 6.4: Pre–shock load batch activity in flocculated biomass culture 1 determined in two sequential batch degradations. This is the initial portion (T < 50 h) of the data of Figure 6.1 presented on a magnified scale in order to illustrate the 0 – order kinetic behaviour.

F = pulse feeding at non–inhibitory phenol level (*ie* < 300 mg/L).

TABLE 6.1: PRE–SHOCK LOAD PHENOL–DEGRADING ACTIVITIES IN FLOC CULTURES

CULTURE	1	2	3
1st BATCH DEGRADATION			
ACTIVITY (mg phenol/L/h)	8.40	8.39	8.44
CORRELATION OF LINEAR FIT	0.9997	0.9998	0.9993
2nd BATCH DEGRADATION			
ACTIVITY (mg phenol/L/h)	7.92	8.33	8.46
CORRELATION OF LINEAR FIT	0.9999	0.9997	0.9999
AVG. ACTIVITY (mg phenol/L/h)	8.2	8.4	8.5

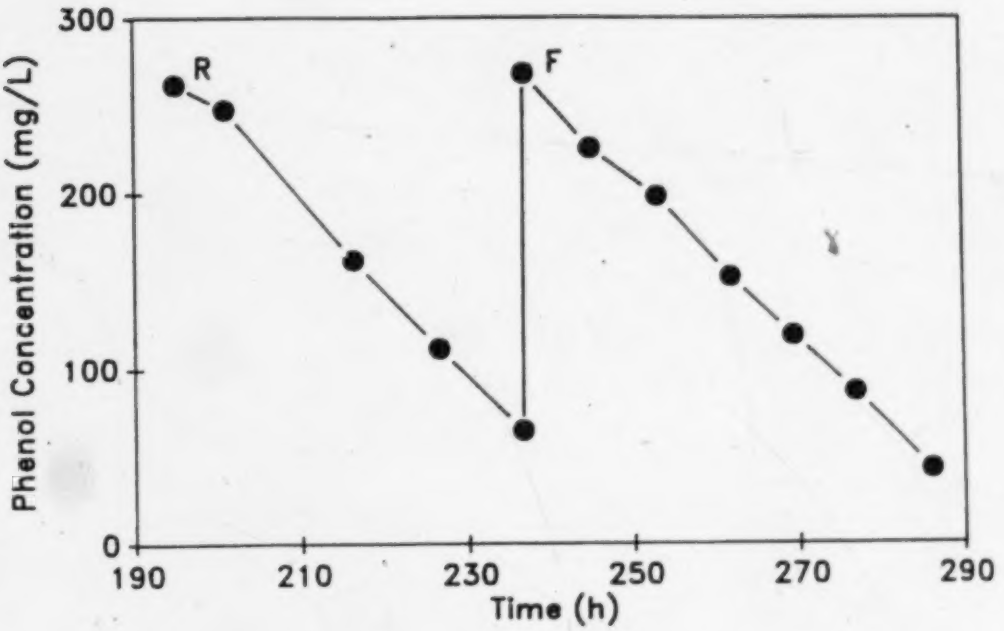


FIGURE 6.5: Post–shock load batch activity in flocculated biomass culture 1 determined in two sequential batch degradations. This is the final portion (T > 190 h) of the data of Figure 6.1 presented on a magnified scale in order to illustrate the 0 – order kinetic behaviour.

F = pulse feeding at non–inhibitory phenol level (*i.e* < 300 mg/L).

R = phenol concentration remaining following degradation of phenol added with shock load.

TABLE 6.2: POST–SHOCK LOAD PHENOL–DEGRADING ACTIVITIES IN FLOC CULTURES

CULTURE	1	2
1st BATCH DEGRADATION		
ACTIVITY (mg phenol/L/h)	4.94	3.73
CORRELATION OF LINEAR FIT	0.998	0.998
2nd BATCH DEGRADATION		
ACTIVITY (mg phenol/L/h)	4.56	4.17
CORRELATION OF LINEAR FIT	0.9994	0.9998
AVG. ACTIVITY (mg phenol/L/h)	4.8	4.0

Notes:

– no post–shock activity observed in FLOC culture 3

TABLE 6.3: SPECIFIC PHENOL–DEGRADING ACTIVITIES IN SHOCK LOADED FLOC CULTURES

CULTURE	1	2	3
SHOCK LOAD (mg/L)[1]	980	1430	1850
PRE–SHOCK			
VS CONTENT (mg)	40	40	40
ACTIVITY[2]	0.21	0.21	0.21
POST SHOCK			
VS CONTENT (mg)	44	45	33
ACTIVITY[2]	0.11	0.097	0
% ACTIVITY REMAINING	58	49	0

Notes:

1. concentration of phenol in supernatant liquid
2. specific phenol–degrading activity in mg phenol/mg VSS/d

sample of the reactor tank contents taken at the same time as inoculation of the serum bottles, the dilution of the culture and the final culture volume. A VSS determination of the serum bottle contents after the completion of the shock loading experiments was used to calculate the post–shock specific degradation rates. In cultures 1 and 2 respectively, 58% and 49% of the initial phenol–degrading activity remained following the shock load. In culture 3, no activity remained. The slightly higher VS content of cultures 1 and 2 is consistent with normal biomass growth. The lower final VS content in culture 3 is indicative of cell death and subsequent breakdown and solubilization of cell material.

The decrease in phenol–degrading activity experienced in cultures 1 and 2 was of a similar order of magnitude and could be a result of two things. The first possibility is a loss of a portion of viable culture resulting from the toxic effects of high phenol concentrations. Concentration gradients within the particles may have protected a large portion of the active culture. Because little information is available on the diffusivities of substrates through bacterial flocs in general, and the coefficient for the diffusion of phenol through the particular flocs present in the cultures studied here certainly is not known, this cause is speculative at best. The second, and more easily supported, explanation is that a high build–up of acetic acid caused a decrease in the activity of the phenol–degrading organisms in the consortium via a product feedback mechanism. A high concentration of acetic acid following the shock load would be consistent with the high acetic acid concentrations observed in the CFRB experiments. The objective of the experiment was to determine the effect of the shock loadings on phenol degradation rates, therefore, acetic acid concentration was not monitored. Acetic acid analysis by the GC method of Section 4.5 was not appropriate for these experiments because the samples required for the acidification step are too large compared to the volume of

the serum bottle cultures.

The net cumulative gas production (gas production measured in the control was subtracted from that of the cultures to yield the net production) shown in Figure 6.6 indicates that methanogenic activity in cultures 1 and 2 continued at more or less a steady rate following the shock loads. Linear regression analyses of the data of Figure 6.6 between the times 100 h and 400 h yielded final post–shock gas production rates of 0.20, 0.16 and 0.02 mL/h for cultures 1, 2 and 3, respectively (correlation coefficients: 0.998, 0.98 and 0.96, respectively). The lower gas production in culture 2 may have been result of lower initial methanogenic activity as well as partial inhibition of the methanogens due to the high phenol concentrations. Unfortunately, the gas production data collected during the pre–shock phase of the experiments was not sufficient to establish reliable pre–shock production rates which would have been useful in a comparison of pre–shock and post–shock methanogenic activity. Note that while phenol degradation in culture 1 was complete within 300 h, gas production continued until almost 400 h following the start of the experiment. This fact strongly supports the case for a large build–up of acetic acid which remained to be degraded after the phenol had disappeared and is a further illustration of the rate–limiting role of the methanogenic population in the phenol–degrading consortium.

Acetic acid concentrations were determined 600 h after the initiation of the experiment. No acetic acid was detected in cultures 1 and 3 and a low level, *e.g.* 10 mg/L, was detected in culture 2. After 600 h, the acetic acid built–up in cultures 1 and 2 would have had been degraded. According to Figure 6.6, gas production in culture 3 continued for approximately 60 h following the shock load and then abruptly decreased ceased and eventually ceased. Combined with the lack of residual acetic acid in the culture, this suggests that the high phenol concentration was completely inhibiting the activity of the phenol–degrading

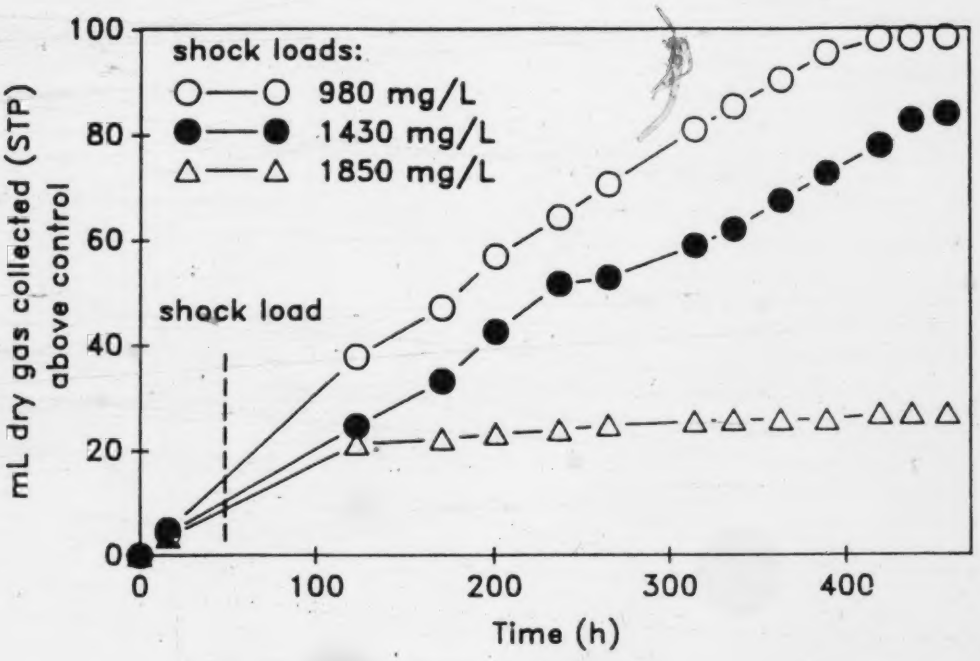


FIGURE 6.6: Measured cumulative net dry gas production in shock loaded flocculated biomass cultures (pre and post–shock load)
open circles – FLOC Culture 1: 980 mg/L shock load
filled circles – FLOC Culture 2: 1430 mg/L shock load
open triangles – FLOC Culture 3: 1850 mg/L shock load

organisms. However, sufficient methanogenic activity remained to remove the acetic acid generated during the pre–shock loading degradation.

These findings suggest that two different mechanisms exist which dictate the response of phenol–degrading cultures to stresses of high phenol concentration. At lower levels of stress, *i.e.* phenol concentrations from approximately 980 mg/L to 1430 mg/L, the activity of the phenol–degrading organisms is limited by product concentration feedback inhibition. In other words, a build–up of acetic acid intermediate in the batch–cultures causes product inhibition of the phenol oxidation reaction. In a continuous culture, acetic acid build–up would be less severe because of continuous wash–out. Substrate inhibition of acetate degradation by the methanogens was not expected at the concentrations of acetic acid that the cultures would have experienced. Acetoclastic methanogens can tolerate much higher concentrations of acetic acid. For example, Yang and Okos (1987) reported maximum growth rates of three pure strains of acetoclastic methanogens occurring at acetate concentrations of 3 600 mg/L, or greater, at a pH of 7.

Above a threshold concentration, phenol itself becomes strongly inhibitory or even toxic to the phenol–degraders. For the cultures in this study, that threshold is somewhere between 1430 mg/L and 1850 mg/L. The activity of the methanogenic organisms does not appear to be directly affected by the high phenol concentrations to a great extent. Phenol inhibition seems to primarily affect the phenol–degrading organisms. This agrees with observations made by Fedorak and Hrudey (1984b) and Wang *et al.* (1989). A more complete study would be required to confirm these hypotheses concerning the mechanisms of inhibition.

6.3.2 ADSORPTION ISOTHERMS

The isotherms for the adsorption of phenol onto clean GAC and GAC from the bioreactors are shown on Figure 6.7. The curves represent best fits of the

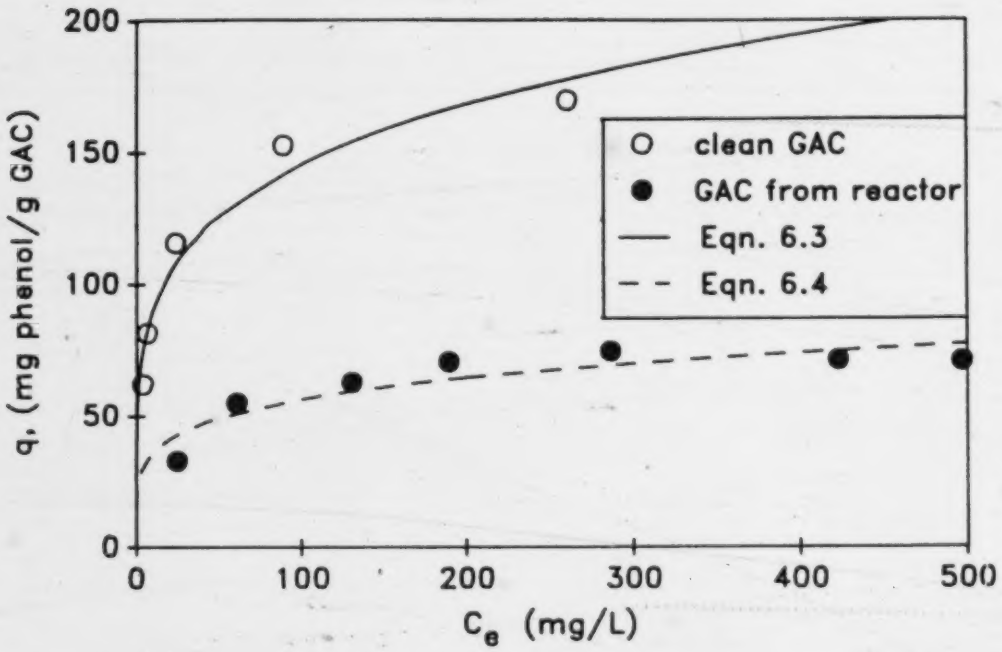


FIGURE 6.7: Data for phenol adsorption isotherms determined for GAC from the bioreactor column and clean F300 GAC. Curves shown are best fits of the Freundlich isotherm (equation 6.2) determined by a nonlinear regression routine. Best–fit isotherms for the clean GAC and the GAC from the bioreactor column are described by equations 6.3 and 6.4, respectively.

Freundlich isotherm equation to the equilibrium data. The Freundlich equation has the general form:

$$q = k\, C_e^{1/n} \qquad (6.2)$$

where:

q is mg adsorbed phenol per g of GAC,

C_e is the equilibrium phenol concentration in the supernatant (mg/L),

k, 1/n are equation constants.

The pre–exponential constant, k, is a measure of the adsorption capacity of the GAC. The exponential constant, 1/n, is directly proportional to the sensitivity of the adsorption to the phenol concentration. k and 1/n were determined for both isotherms by a derivative–free non–linear regression routine (BMDP Statistical Software Inc., L.A., Cal.).

The Freundlich equations determined for the clean GAC and the GAC from the bioreactor were, respectively:

$$q = 55.4\, C_e^{0.21} \qquad (6.3)$$

and

$$q = 22.8\, C_e^{0.20} \qquad (6.4)$$

The sensitivities are approximately the same, however, the adsorption capacity of the GAC from the bioreactor has been reduced by 59%. Kindzierski (1989) observed a reduction in adsorption capacity of 47% in a study of phenol adsorption kinetics with GAC in anaerobic serum bottle cultures. Blockage of access to a portion of the micropore system of the carbon by cells and cell associated material (*e.g.* exopolysaccharide) may account for a significant proportion of the observed reduction in capacity. Adsorption of medium constituents, rezasurin indicator for

example, will also hinder phenol adsorption by competing for available sites on the GAC. Another possible reason for the reduction in phenol adsorption capacity indicated by the data, which must be considered, is incomplete desorption during preparation of the GAC for the assay. Although no phenol was detected by a GC analysis of the supernatant after 24 h of equilibrating and prior to phenol addition, it is possible that an unknown quantity of pre–adsorbed phenol remained on the GAC. Figure 6.7 shows that the isotherm is extremely steep at very low concentrations. Complete desorption of phenol by heating of the GAC at 105^{o}C for 48 h cannot be guaranteed. To be conservative, therefore, equation 6.3 should be considered to be a lower bound on the adsorption of phenol. The true phenol adsorption isotherm for the GAC from the reactor probably lies somewhere between the two curves of Figure 6.7.

6.3.3 SHOCK LOADING OF GAC CULTURES

Exact reproduction of the maximum concentrations in the supernatant, experienced by the flocculated biomass cultures, in the GAC culture shock loading experiments was difficult. Supernatant phenol concentrations were below 300 mg/L during determination of the pre–shock degradation activity. The concentrations following shock loading were 1130 mg/L, 1540 mg/L and 2170 mg/L in GAC cultures 1, 2 and 3 respectively. The bulk of the phenol added with the pulse feeding was rapidly adsorbed onto the GAC. This was taken into account in determining the quantities of phenol to be added. To avoid exposing the culture to extremely high spikes of phenol concentration in the short time period immediately after pulse feeding and prior to bulk adsorption, the shock loads were applied as several small pulse feedings over a 2 day period. Strictly speaking, they, therefore, were not true shock loads.

The responses of the three cultures to the shock loads are shown in

Figures 6.8, 6.9 and 6.10. Culture 1, the least stressed culture, exhibited undelayed and complete degradation of the phenol added. The responses of the more stressed cultures were much slower. In order to quantitatively compare pre–shock load and post–shock load phenol–degrading activities, the concentration versus time data of Figures 6.8, 6.9 and 6.10 alone was insufficient. Calculation of phenol degradation rates using the phenol adsorption equilibrium information was necessary.

The total amount of phenol in the cultures was the sum of the adsorbed phenol and the phenol in the supernatant liquid and was determined by the following equation:

$$m_p = C_e V_l + m_c q \qquad (6.5)$$

where:

m_p is the total mass of phenol (mg),

C_e is the equilibrium phenol concentration (mg/L),

V_l is the culture liquid volume (mL),

m_c is the mass of the GAC (g),

q is adsorbed phenol per gram GAC (given by equations 6.3 or 6.4).

Plotting m_p versus time yields the biological phenol–degrading activity of the culture. As an example, the results of the analysis of the pre–shock load and post–shock load activities of culture 1 are illustrated in Figures 6.11 and 6.12, respectively. Similar figures for cultures 2 and 3 are found in Appendix III. Estimates of total phenol determined by application of both isotherm expressions (equation 6.3 and equation 6.4) are shown in these figures. Upper and lower bounds on the specific degradation activity respectively, were determined by linear least–squares analyses of these data. Table 6.4 summarizes the estimated pre–shock activities. Correlations of linear fit are given. Degradation activities in

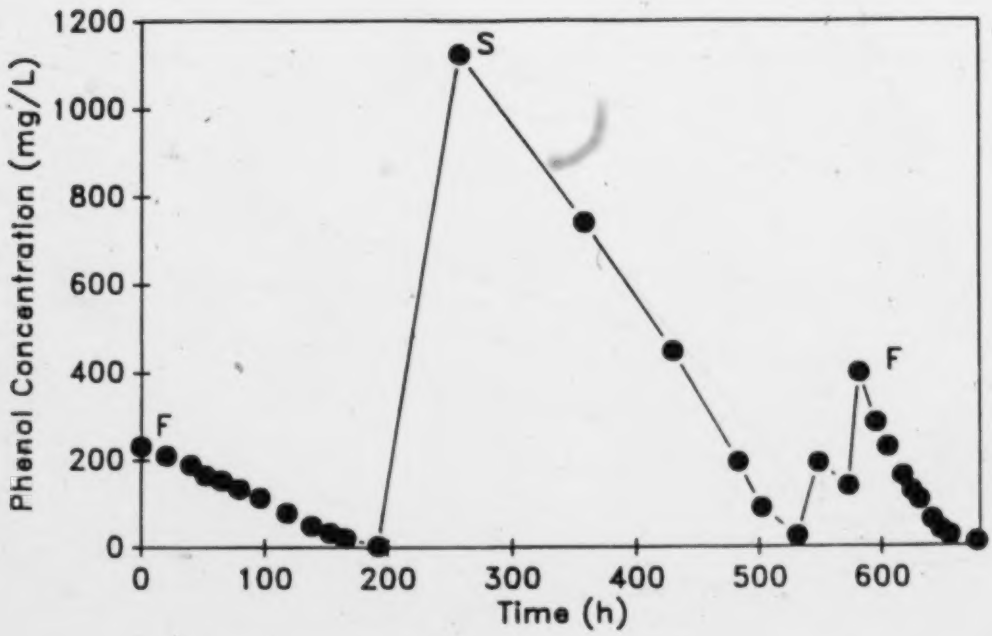


FIGURE 6.8: Response of GAC culture 1 to a phenol shock load of 1130 mg/L in the supernatant. Pre–shock batch activity was determined in a single batch degradation at low phenol concentration. Post–shock batch activity was verified by a final batch activity determination.

F = pulse feeding at non–inhibitory phenol level (*i.e* < 300 mg/L in the supernatant).

S = shock load pulse feeding to inhibitory phenol level.

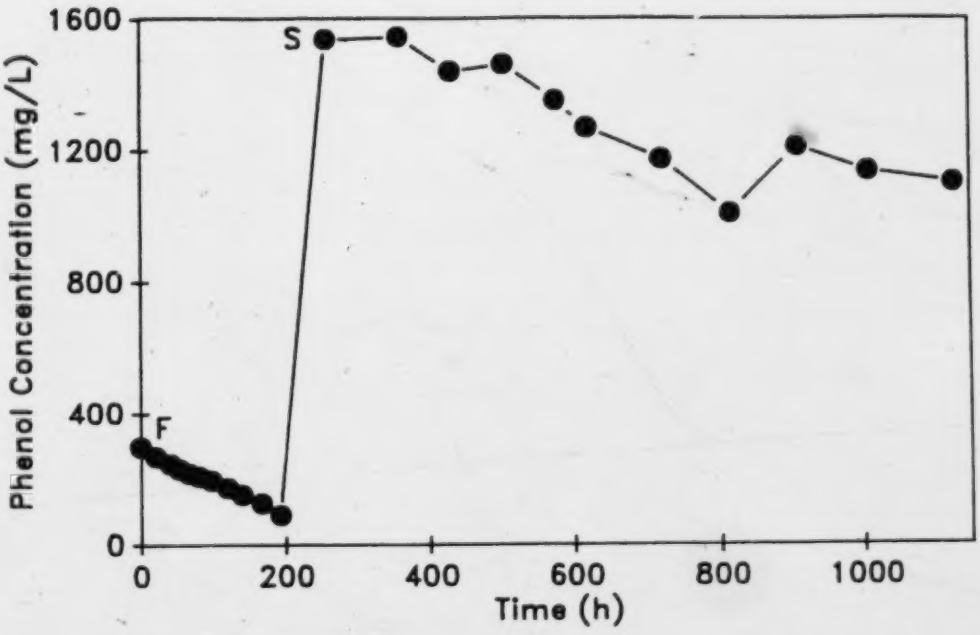


FIGURE 6.9: Response of GAC culture 2 to a phenol shock load of 1540 mg/L in the supernatant. Pre–shock batch activity was determined in a single batch degradation at low phenol concentration. No post–shock feeding was performed because the supernatant phenol concentration remained at inhibitory levels.

F = pulse feeding at non–inhibitory phenol level (*i.e* < 300 mg/L in the supernatant).

S = shock load pulse feeding to inhibitory phenol level.

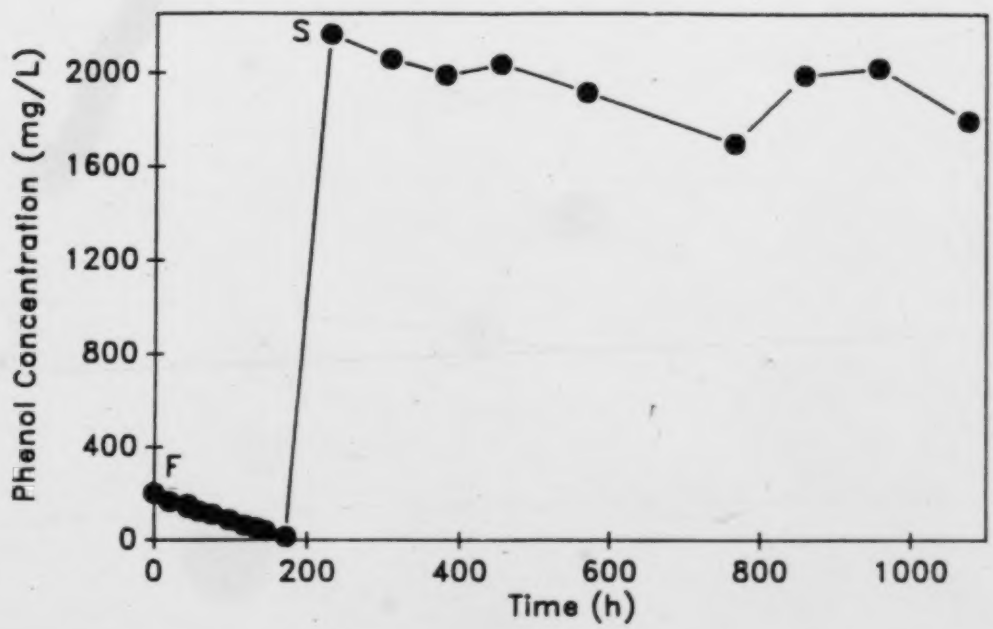


FIGURE 6.10: Response of GAC culture 3 to a phenol shock load of 2170 mg/L in the supernatant. Pre–shock batch activity was determined in a single batch degradation at low phenol concentration. No post–shock feeding was performed because the supernatant phenol concentration remained at inhibitory levels.

F = pulse feeding at non–inhibitory phenol level (*i.e* < 300 mg/L in the supernatant).

S = shock load pulse feeding to inhibitory phenol level.

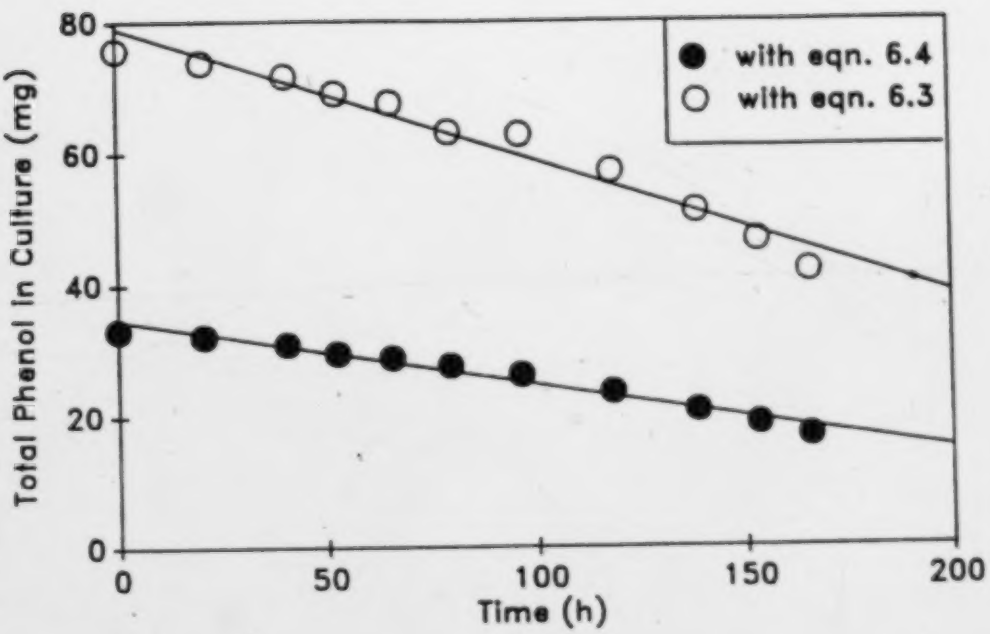


FIGURE 6.11: Pre–shock load activity in GAC culture 1. Total phenol in the culture (sum of adsorbed phenol and phenol in supernatant) is on the vertical axis. Top curve is upper bound on total phenol calculated using the experimentally determined phenol adsorption isotherm for clean GAC (equation 6.3). Bottom curve is lower bound on total phenol calculated using the experimentally detemined phenol adsorption isotherm for GAC from the bioreactor column (equation 6.4). Linear least–square fits to the data are shown.

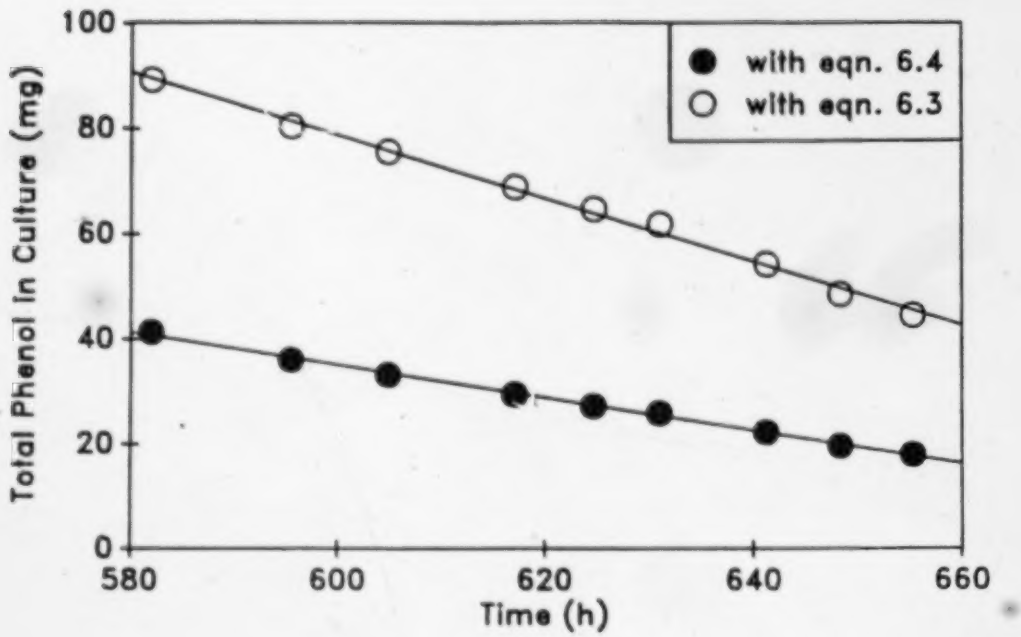


FIGURE 6.12: Post–shock load activity in GAC culture 1. Total phenol in the culture (sum of adsorbed phenol and phenol in supernatant) is on the vertical axis. Top curve is upper bound In total phenol calculated using the experimentally determined phenol adsorption isotherm for clean GAC (equation 6.3). Bottom curve is lower bound on total phenol calculated using the experimentally determined phenol adsorption isotherm for GAC from the bioreactor column (equation 6.4). Linear least–square fits to the data are shown.

TABLE 6.4: SPECIFIC PHENOL–DEGRADING ACTIVITIES IN SHOCK LOADED GAC CULTURES

CULTURE	1	2	3
SHOCK LOAD (mg/L)[1]	1130	1540	2170
PRE–SHOCK ACTIVITY[2]			
UPPER BOUND	0.13 (0.982)[3]	0.07 (0.992)	0.10 (0.993)
LOWER BOUND	0.07 (0.986)	0.04 (0.995)	0.05 (0.996)
POST–SHOCK ACTIVITY[2]			
UPPER BOUND	0.40 (0.998)	0.02 (0.885)	0.008 (0.662)
LOWER BOUND	0.21 (0.999)	0.01 (0.889)	0.006 (0.62)

Notes:

1. concentration of phenol in supernatant liquid
2. based on total phenol in culture and TGA measurement of GAC–attached biomass from bioreactor study (units are mg phenol/mg VS/d)
3. correlations of linear fit are in parentheses (no units)

Table 6.4 are specific activities that are normalized with respect to VS content of the culture. VS content was estimated by determining the mass of dry GAC in each culture (0.396 g, 0.386 g and 0.444 g in cultures 1, 2 and 3, respectively) and assuming the same biomass concentration on the GAC as in the bioreactor study *i.e.*-0.101 g VS/g dry GAC.

The concentration in cultures 2 and 3 remained above 1000 mg/L following the shock load (Figures 6.8 and 6.10). Determinations of post–shock load biological activity were less reliable because the concentrations involved were well outside the range for which the isotherm equations 6.3 and 6.4 were determined (*i.e.* < 300 mg/L and < 500 mg/L, respectively). Figure 6.7 suggests that equations 6.3 and 6.4 would tend to over–estimate the adsorption capacity of the GAC at equilibrium concentrations above 1000 mg/L. A lower bound on the activity at these high concentrations was estimated by assuming that adsorption of phenol onto the GAC was independent of concentration, that is, the GAC was assumed to be saturated. Given this condition, only the quantity of phenol in the supernatant changed with time. Phenol–degrading activity was then estimated directly from the the concentration versus time data of Figures 6.9 and 6.10.

The upper and lower bounds the post–shock degradation rates for the three cultures are summarized in Table 6.4. The upper bounds were determined using equation 6.3, the adsorption isotherm for the clean GAC. The lower bound for culture 1 was determined using equation 6.4 because the concentrations were in the appropriate range for application of the isotherm for the GAC from the reactor. The lower bounds for cultures 2 and 3 were calculated using the adsorption saturation assumption.

A comparison of the pre–shock load and post–shock load activities, listed in Table 6.4, indicates that, following a shock load of 1130 mg/L in culture 1, there was no inhibition of phenol degradation. In fact, the results show an increase in

activity. Part of the observed increase may have been due to a greater content of active biomass in the culture which resulted from cell proliferation. Inhibition of phenol degradation at supernatant phenol concentrations of 1540 mg/L and 2170 mg/L was quite severe and was worse at the higher concentration. These interpretations are supported by the net cumulative gas production of the cultures (Figure 6.13). Gas production in culture 1 increased following the shock load, which is consistent with the observation of greater phenol–degrading activity. The rate of gas production declined after shock loading in culture 2. In culture 3, gas production essentially ceased 100 h following the shock load.

6.3.4 COMPARISON OF GAC AND FLOC CULTURE RESPONSES

A comparison of the results of Tables 6.3 and 6.4 indicates that the pre–shock specific activity of the GAC–attached culture was significantly lower than the pre–shock specific activity of the flocculated biomass cultures. The latter results were consistent with similar results obtained in the bioreactor experiments (*i.e.* 0.19 and 0.18 mg phenol/mg VS/D, Table 5.4). On the other hand, the specific activities of the GAC–attached culture in Table 6.4 are much lower than those obtained in the bioreactor experiments (*i.e.* 0.16 and 0.14 mg/L/d, Table 5.4). A possible explanation for this anomolous result is a partial loss activity during the GAC culture set–up. During the transfer procedure inside the anaerobic chamber, a portion of the attached culture may have been exposed to oxidizing conditions or may have been partially dried out, resulting in a loss of viability. The flocculated culture set–up procedure, on the other hand, was based on established serum bottle techniques and did not require direct exposure of the bacteria to a gaseous atmosphere. These cultures, therefore, were less likely to have been oxidized or dried out. The loss of attached culture activity may have been temporary. This would explain the higher post–shock activity which was observed in GAC culture 1.

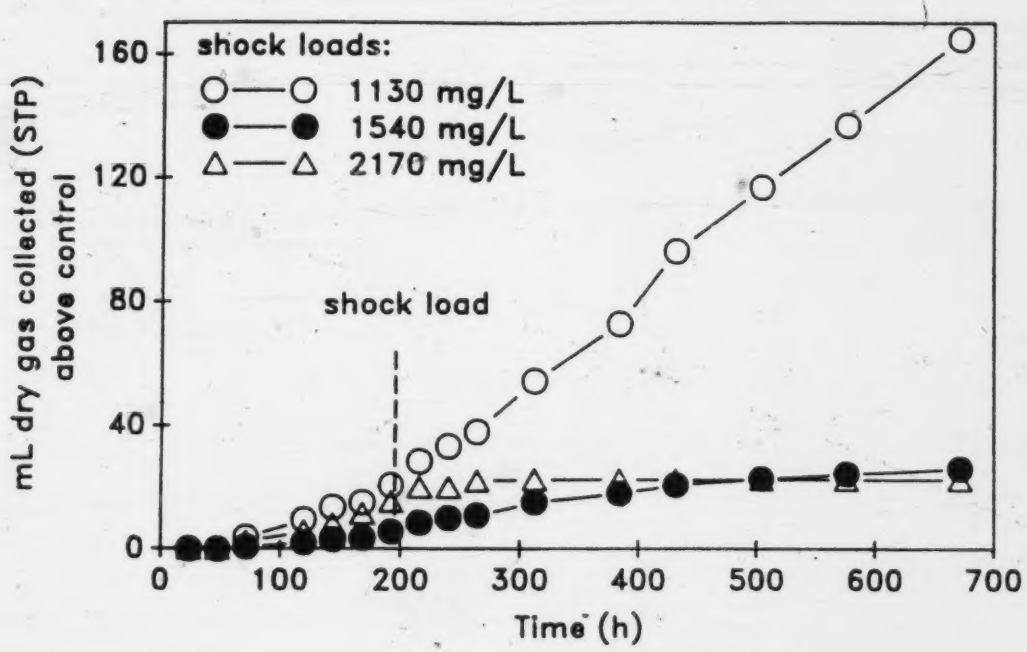


FIGURE 6.13: Measured cumulative net dry gas production in shock loaded GAC cultures (pre and post–shock load).
open circles – GAC Culture 1: 1130 mg/L shock load
filled circles – GAC Culture 2: 1540 mg/L shock load
open triangles – GAC Culture 3: 2170 mg/L shock load

In comparing the responses of the stressed GAC cultures to similarly stressed cultures containing the flocculated biomass some improvement of performance was observed in the GAC cultures, especially at the lower levels of system stress. At a shock loading of 1130 mg/L, neither phenol degradation nor gas production were inhibited in the GAC culture. Following a shock load of 980 mg/L, the phenol–degrading activity of the culture was noticeably reduced, presumably as a result of acetic acid inhibition of the phenol–degrading organisms. The reduction of acetate concentrations by adsorption onto the GAC would explain the better response of the the GAC culture, however, low molecular weight, polar organics are generally poorly adsorbed by GAC (Metcalf and Eddy Inc., 1979). Perhaps the adsorption of acetic acid that does occur was sufficient to reduce the concentration to prevent feedback inhibition of phenol oxidation. The experience of the bioreactors indicated that a certain build–up of acetic can be tolerated before inhibition of phenol degradation is observed. In a review on carbon adsorption in the treatment of industrial wastes, Lyman (1978) stated that acetic acid is generally considered as poorly adsorbable, however, some adsorption is achieved. He reported an acetic acid loading of 40–60 mg/g in the treatment of a phenol–containing, brine wastewater. This capacity would be sufficient to measurably reduce the concentration of acetic acid in the supernatant of the shock loading cultures. Acetic acid was not detected in any of the culture supernatants after termination of the experiment. Verification of this protective mechanism provided by the GAC would require further experimental work on shock loaded cultures, involving acetic acid analyses, and characterization of acetic acid adsorption in this system.

At shock loadings of 1540 mg/L and higher, the response of the GAC culture was not remarkably improved over that of the flocculated biomass culture. The 1540 mg/L GAC culture may have been subjected to a phenol concentration greater then the toxic threshold. GAC culture 3, subject to a 2170 mg/L shock load,

certainly was. A small amount of residual phenol–degrading activity did remain in GAC cultures 2 and 3, which suggests that a small portion of the culture was protected from the bulk liquid phenol concentration by diffusional limitations. There was insufficient experimental evidence to support the contention that diffusional limitations are effective in protecting the GAC–supported culture from inhibitory or toxic concentrations in the bulk liquid. The rate of adsorption of phenol to GAC is much greater than the rate of phenol degradation by the immobilized culture preventing the establishment of concentration gradients by this mechanism.

7 ADDITIONAL STUDIES ON THE GAC AND FLOC CULTURES

7.1 SCANNING ELECTRON MICROSCOPY

7.1.1 GAC PARTICLES FROM BIOREACTOR

Scanning electron microscopy micrographs were taken of GAC particles removed from the bioreactor column after shutdown. Procedures for sample preparation and viewing were described in Section 4.7. Particles were randomly selected for viewing and several were photographed. The column contents were mixed well prior to sampling so that particles were not selected from only one location in the bed. Photographs selected for presentation exhibit characteristics that were typical of all the particles viewed.

Figure 7.1(a), 7.1(b) and 7.2(a) are low magnification micrographs that show extensive colonization of the GAC particles. The surface features of the GAC, the macropores and crevices, appear completely full of dense bacterial growth and associated material. Growth on the exposed, outer surface of the GAC particles is sparse and uneven and extensive bare carbon surface is visible. Chen *et al.* (1988) described complete biofilm formation and went so far as to measure biofilm thickness on GAC particles from an anaerobic fluidized bed reactor treating a corn starch wastewater. In contrast, no complete biofilm was observed in the phenol–degrading expanded bed system of the present study even after 276 d of growth.

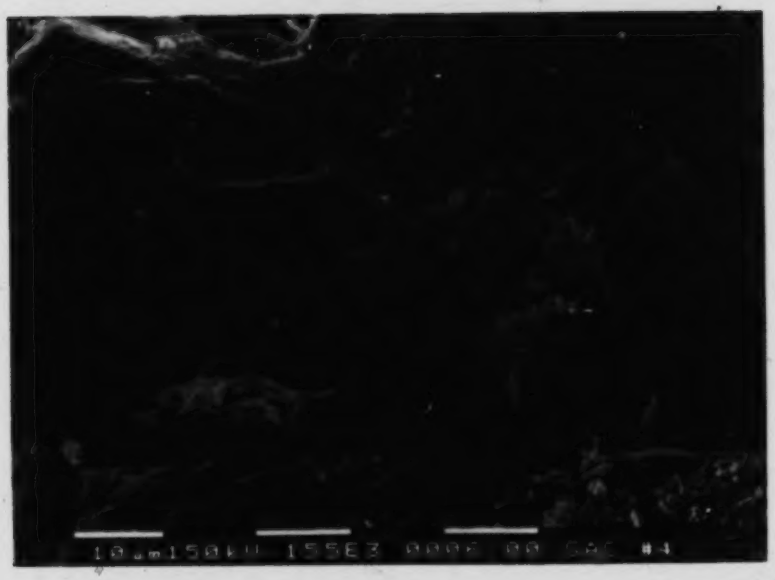
Kindzierski (1989) also reported lack of complete biofilm formation. In an S.E.M. study, he observed almost no surface growth on GAC particles removed from anaerobic, phenol–degrading serum bottle cultures but he did observe massive growth in the surface pores and crevices. Removal of biomass from exposed surfaces by the mechanism of inter–particle abrasion would be much greater in constantly

(a) Bar = 100 μm

(b) Bar = 100 μm

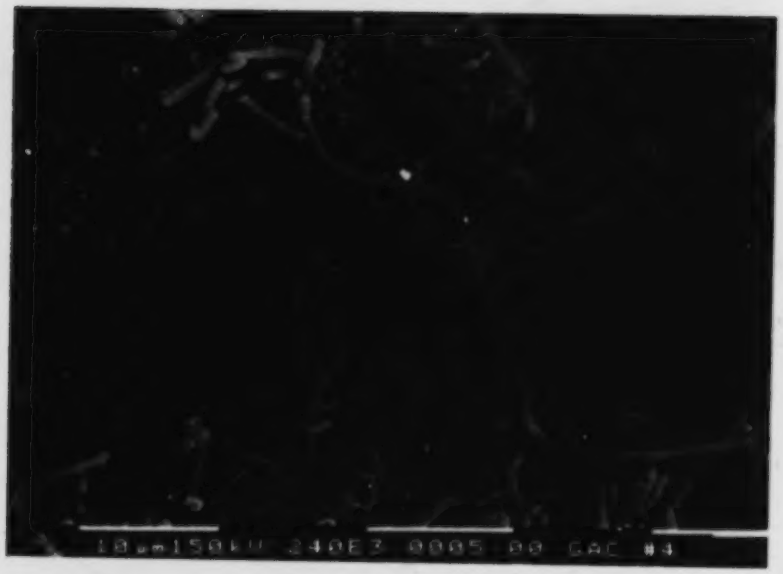
FIGURE 7.1: **Colonized GAC from Bioreactor**

(a) Bar = 100 μm



(b) Bar = 10 μm

FIGURE 7.2: **Colonized GAC from Bioreactor**



(a) Bar = 10 μm

(b) Bar = 10 μm

FIGURE 7.3: Colonized GAC from Bioreactor

shaken serum bottles employed by Kindzierski than in the expanded bed reactor of the present study. Particles in an expanded bed remain stationary relative to each other and, therefore, inter–particle abrasion is minimal. Although the GAC in Kindzierski's study had been exposed to growth for only 100 d, compared to 276 d in the bioreactor, the observed re–seeding phenomena indicated that the biomass growth on the activated carbon had stabilized and was balanced by the action of removal mechanisms

Hydraulic shear and periodic agitation, resulting from the release of gas produced within the bed, are the mechanisms which most likely account for constant biomass removal from the GAC particles in the expanded–bed reactor. Constant removal of biomass balances growth and accounts for the lack of complete biofilm formation and also for the re–seeding phenomena. Figures 7.1(b) and 7.2(b) are excellent illustrations of the preference of the culture for the sheltered regions of the GAC surface, where the effects of hydraulic shear and agitation are minimized, and the sparse growth on the exposed surfaces.

Morphological characteristics of the attached culture are illustrated in the high magnification micrographs of Figures 7.3(a) and 7.3(b). Although the complex and non–homogeneous nature of the biomass is evident, two types of filamentous organisms tend to predominate. A larger diameter organism, with distinctive flat ends is similar to the acetate–utilizing *Methanothrix* genus of methanogens. A much thinner, string–like organism may be a hydrogen–utilizing methanogen. Both types of filamentous organisms are present in a wide variety of lengths (Figure 7.3(a)). In addition, there are a comparatively smaller number of small, oval rods which are the proposed phenol–oxidizing bacteria. The tentative description of a three member consortium is consistent with the S.E.M. observations made by Dwyer *et al.* (1986). Figures 7.1 to 7.3 all indicate the presence of a significant amount of material closely associated with the organisms. This material,

which exhibits no characteristics of cellular organisms or of the GAC support matrix, is presumably a glycocalyx composed of organic polymer excreted by the bacterial cells. This fibrous–like exo–polymer appears to play an important role in firm attachment by acting as a support matrix and binding agent for the organisms.

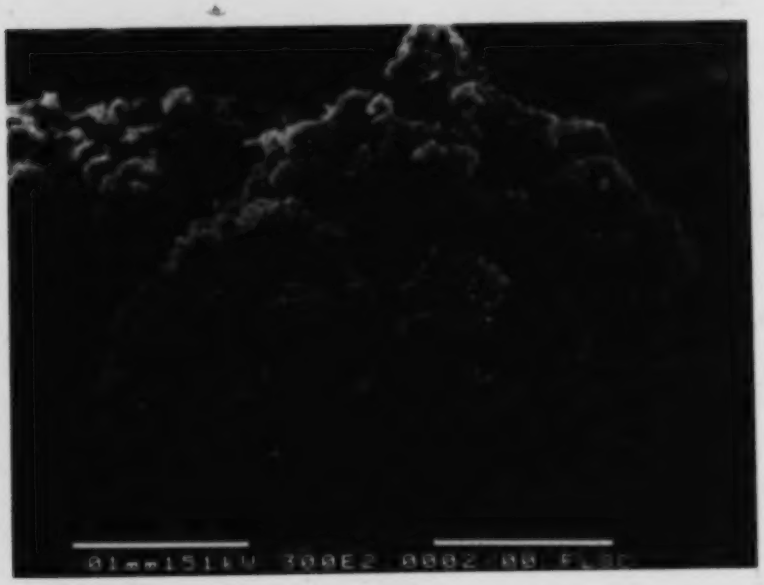
7.1.2 FLOCCULATED BIOMASS FROM BIOREACTOR

A sample of flocculated biomass was removed from the bioreactor tank at the time of shut–down for S.E.M. viewing. It was desired to view the larger floc particles to determine if granulation was occurring. Smaller particles of biomass were separated from the larger, faster settling particles by a series of sedimentation steps performed in test tubes.

The S.E.M. photographs (Figures 7.4, 7.5 and 7.6) indicate that distinct particles were forming in the reactor. The biomass particles appear very dense and well–defined as opposed to loosely associated flocs. Figures 7.4(a), 7.4(b) and 7.5(a) show very distinct, separate particles of approximately 0.3 mm in size. Although they were much smaller then the 2 mm to 5 mm particle sizes that have been reported by other workers studying granulation in UASB reactors (*e.g.* Hulshof Pol *et al.*, 1983), these do suggest the onset of granulation in the phenol–degrading consortium. This is the first report of granulation in cultures receiving phenol as the major carbon source.

The predominance of the the filamentous, *Methanothrix*–like, organisms comprising the granules is shown in the higher magnification micrographs of Figures 7.5(b), 7.6(a) and 7.6(b). Of the granule types identified by Hulshof Pol *et al.* (1983), these granules most closely resemble the filamentous type. Compared to the the culture supported by the GAC described in the previous section, there are fewer of the thinner, string–like, filamentous organisms and almost a complete absence of small, oval rods on the exterior of the granules. These organisms were tentatively

(a) Bar = 100 μm



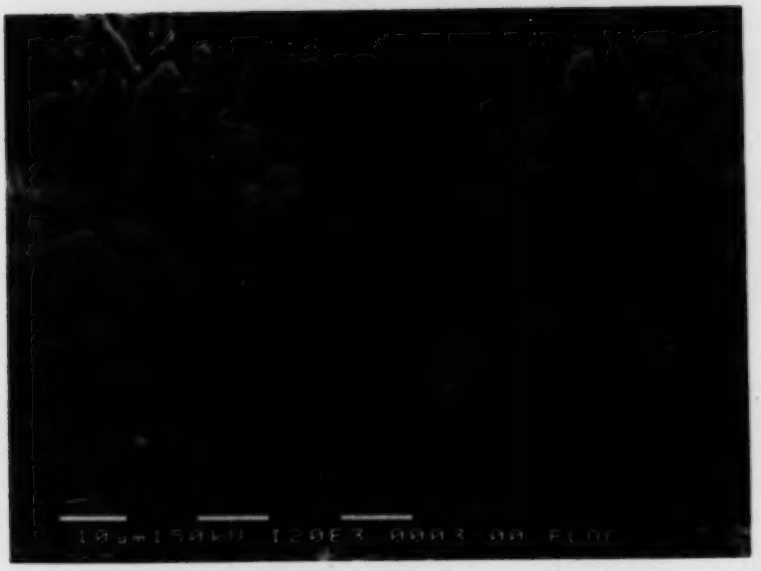
(b) Bar = 100 μm

FIGURE 7.4: **Granular Biomass From Bioreactor**

(a) Bar = 100 μm

(b) Bar = 100 μm

FIGURE 7.5: Granular Biomass From Bioreactor



(a) Bar = 10 μm

(b) Bar = 10 μm

FIGURE 7.6: **Granular Biomass From Bioreactor**

identified as the H_2–utilizing methanogens and the phenol–oxidizers, respectively. Extra–cellular polymer material was observed to be an integral part of the granule make–up. The presence of extensive extra–cellular polymer is especially well illustrated in Figures 7.5(b) and 7.6(b) in which the organisms appear tightly embedded in a polymer matrix that is holding the particles together.

Figure 7.6(a) best illustrates the incorporation of what resembles inorganic solid particles into the granule matrix. In this system, these solid particles were expected to be GAC fines or iron sulphide precipitate. A dispersive X–ray scan was performed on a sample of the flocculated biomass which was observed to contain these solid particles. The scan was performed on the scanning electron microscope in the Department of Mineral Engineering at the University of Alberta, Edmonton, Alberta. Iron sulphide would have been identified by strong signals characteristic of the elements iron and sulpher, however, no such signals were detected. An elemental analysis of the flocculated biomass from the reactor resulted in a significantly higher carbon content than what was determined for the culture from the GAC–free 2–L digester which served as the reactor inoculum (see Section 7.2.6). The conclusion, therefore, is that the unidentified solid particles were GAC fines. It is of interest to know what role these solids play in the formation of granules. Incorporation of the solids would serve to increase the effective granule density and would, therefore, promote settling and retention of the biomass particles. Another important and unanswered question regards the role that the GAC fines played in the initiation of granule formation. That is, did the carbon fines play an active role in promoting granule formation by serving as points of nucleation or were the fines randomly incorporated into the particle matrix?

7.1.3 SUMMARY AND DISCUSSION

The most important observation of the GAC–supported culture provided by

S.E.M. micrographs that, even at conditions of low inter–particle abrasion experienced in the expanded–bed reactor, the bacterial growth was most concentrated in the surface pores and crevices of the GAC. In the phenol–degrading system, no complete biofilm was oserved. Under conditions which are similar to those experienced in full–size treatment reactors, *e.g.* expanded bed operation, the irregularity of the GAC surface remains a vital feature of its performance as a support media.

The S.E.M. micrographs of the flocculated biomass indicated the onset of granulation although the size of the granules viewed was small in light of reports of granule formation in other systems. The stabilization of a bed of this biomass above the GAC bed in the bioreactor column indicates that, even at this small size, the biomass particles were highly resistant to wash–out. An extended period of operation would have resulted in continued increase in granule size due to growth unless mechanisms, such as hydraulic shear, were limiting the size of the granules. Extracellular polymer appears to play an important role in granule formation by serving as type of support matrix to which the bacteria are tightly bound. Inert particles, most probably activated carbon fines, were incorporated into the matrix, however, it is not known if these particles actively participated in granule formation.

A comparison of the S.E.M. micrographs of the GAC and floc particles shows that the granules formed were physically quite similar to the cultures supported inside the large surface pores of the GAC. Both culture types appeared quite dense and are characterized by a matrix composed of extracellular polymeric material. The size of the colony located in the large pore at the center of the GAC particle shown in Figure 7.1(a) was approximately 0.2 mm which is about the same size as the granules observed in Figures 7.4(a), 7.4(b) and 7.4(c). The surface pores of the GAC may promote granule formation by serving as a sheltered location for the

initial growth of the granules. As the granules out–grow the capacity of the pores which they occupy they are sloughed off by removal mechanisms and released into the medium. Conversely, the granules may begin to form in the supernatant before finding a comfortable location for attachment inside the surface macropores.

The apparent difference in the concentration of morphological types between the attached culture and the floc–granule culture is potentially important to the design of an attached growth treatment reactor. An effective treatment system requires that a complete and balanced consortium be maintained. Although all the morphological types of the tentatively described 3–member phenol–degrading consortium were identified within the GAC supported culture, far fewer proposed H_2–utilizing methanogens and almost no proposed phenol–oxidizing bacteria were observed on the surface of the granules. A possible explanation for the lack of phenol–oxidizers is that these bacteria require the low shear environment of the GAC surface features to bind to the rest of the consortium. The hydraulic shear environment at the surface of the larger granule particles may have prevented attachment of the phenol–oxidizers. Recall from the shock–loading experiments on the flocculated cultures (Chapter 6) that phenol was initially readily degraded at a rate similar to that experienced in the CFRB experiments. These cultures were composed not only of the larger, granular biomass particles but also of the lighter, suspended biomass as well as reactor supernatant. The phenol–oxidizers may have been concentrated in the supernatant liquid or in the smaller biomass particles. They may also have been concentrated in the interior of the larger particles and, therefore, were not visible by S.E.M. If this explanation was valid, it suggests that the granule size is limited by shear forces. That is, shear forces on the particle increase with particle size until a point is reached where the surface can no longer support a complete phenol–degrading consortium and, therefore, there is no metabolic incentive for further growth. It may also explain the superior

performance of the GAC–supported cultures over the flocculated cultures at the low shock loadings. The proximity of the phenol–oxidizers to the methanogens, observed in the supported culture, increases the efficiency of inter–species transfer of inhibitory intermediates (*e.g.* acetic acid, H_2) and, therefore, the efficiency of their degradation. An increase in the efficiency of degradation of these intermediates will lower their concentration and thereby reduce the effects of feedback inhibition.

S.E.M. studies provide valuable qualitative information on the characteristics of cultures and supports, however, they are limited and the observations must be interpreted with caution. S.E.M. allows observation of only the surface of a particle and provides no information about the interior. For instance, the S.E.M. micrographs did not indicate the depth to which the biomass impregnated the pore system of the GAC. It is not possible to characterize the morphology of the interior of the granular particles with S.E.M. Although it suggests the existence of certain genera or species of bacteria, S.E.M. alone is not sufficient to make positive microbial identifications. When interpreting information in S.E.M. photographs, consideration must be given to the possibility of the production of artifacts, especially during the sample preparation procedure. For instance, the absence of biological growth on parts of the GAC surface may be a result of the removal of this biomass during the preparation procedure. Chang and Rittman (1986) reported detachment of aerobic biofilms from glass beads subject to the conventional set of S.E.M. sample preparation steps (*i.e.* fixation, postfixation, dehydration, mounting and coating). Biofilm removal was reduced by using a modified preparation procedure which involved sandwhiching the samples between filter membranes during fixation and dehydration. An experiment could be designed to check for removal of anaerobic biofilm from GAC particles. GAC particles colonized with radiolabelled cells would be subject to the normal S.E.M. preparation

procedure. Biofilm loss would be indicated by detection of radioactivity in the spent preparation solutions.

7.2 THERMOOGRAVIMETRIC ANALYSIS

7.2.1 BIOMASS MEASUREMENT

Producing a reliable estimate of the quantity of biomass attached to GAC in the phenol–degrading anaerobic system was not straightforward. The proportion of biomass on the GAC carrier is relatively small and sensitive quantification is difficult. In this study, the concentration of volatile solids on the GAC was about 10%. Techniques that do not differentiate between cell associated organics and non–cell associated organics, like the standard VSS determination (described in Section 4.8) or elemental analysis, are complicated by the tendency of the carbon to adsorb significant quantities of organics from the medium. Phenol is an example. Accurate accounting for the amount of organics adsorbed, like phenol, is often not possible. The use of the standard VSS procedure is further aggravated by weight changes caused by oxidation of the GAC during high temperature (550°C) firing of the samples. Senthilnathan and Ganczarczyk (1988) describe a similar problem encountered in studies of aerobic, activated sludge processes which employ carbonaceous carriers.

Standard techniques which are specific for cellular materials, such as ATP, protein, lipids, carbohydrates or organic nitrogen are often useless in systems employing GAC. These methods generally involve colorimetric reactions and spectrophotometric absorption measurements. GAC tends to adsorb the reagents dyes used and to interfere with the adsorption measurements. Furthermore, Kindzierski (1989) found that organic (Kjeldhal) nitrogen, carbohydrate and protein analyses were inadequate for the measurement of biomass quantities in anaerobic

sludge even in the absence of GAC. Reproducibility was poor and low biomass concentration in the medium was cited as the cause of the difficulty.

An alternate strategy to direct determination methods involves the removal and separation of the attached biomass from the GAC by a physical or chemical pre–treatment. The removed biomass is collected and quantified by a standard technique such as VSS, protein, etc. The use of ultra–sound energy or treatment with solutions of NaOH or sodium dodecyl sulfate are examples of removal procedures. The major problem is the uncertainty of the efficiency of removal. Treatments, like those mentioned above, usually disrupt the cells and breakdown cell components. The smaller fragments may then be rapidly adsorbed onto the GAC. Chen *et al.* (1988) used treatment with a hot NaOH solution to remove anaerobic biofilms from GAC, followed by a standard VSS determination of the wash liquor. They did attempt to verify removal by scanning electron microscopic observation of the washed GAC, however, S.E.M. only provides a visual check of the surface features of the GAC and yields no information on the internal surface. The effect of cell breakdown and solubilization, resulting from the NaOH treatment process, on the standard VSS determination was not considered.

Thermogravimetric analysis, TGA, was employed in a modification of the standard VSS technique. TGA gave a direct estimate of the proportion of attached biomass, as volatile solids, on the GAC removed from the bioreactor. The problem of volatilization of the carbon was minimized by performing the analysis under a non–oxidative (N_2) atmosphere. Weight loss due to pre–adsorbed material and any volatilization of the GAC that did occur was accounted for by incorporating into the calculations a reference TGA determination of an appropriate GAC control. A correction for the difference in the degree of biomass volatilization under N_2 and air atmospheres was estimated so that the TGA result could be converted to the same basis as the standard VSS determination. The unique capabilities of the TGA

apparatus were ideally suited to the sensitive determinations required for the method. All gravimetric determinations were made with good precision and *in situ*, under carefully controlled conditions. Transfers of sample between different ovens and balances were eliminated.

7.2.2 PROCEDURES

The complete procedure for the estimation of the biomass concentration on the GAC from the bioreactor by TGA consisted of the following determinations:

1. Percent weight loss of samples of colonized GAC.
2. Percent weight loss of biomass–free, control GAC.
3. Correction factor for the conversion to a standard VSS basis.

Steps 1 and 2 above were performed under a non–oxidative atmosphere to minimize the weight loss of the carbon itself. The gas employed was pre–purified grade nitrogen. The control GAC was intended to represent the sample GAC from the reactor except for colonization by anaerobic biomass. The correction factor was estimated by performing the identical TGA analysis on a sample of a GAC–free, anaerobic biomass standard under an N_2 atmosphere and then repeating the determination under an atmosphere of standard grade air.

The temperature program employed for the determinations was intended to reproduce the standard VSS technique (Section 4.8) with a few minor modifications. The System 4 Microprocessor Controller was programmed to implement the following temperature profile:

START:	$T_0 = 50^\circ C$	TIME: 2 min
HEAT:	RATE = $50^\circ C$/min	TIME: 1.2 min
HOLD:	$T_1 = 110^\circ C$	TIME: 30 min
HEAT:	RATE = $50^\circ C$/min	TIME: 9 min

HOLD: $T_2 = 560^{\circ}C$ TIME: 30 min

The holding temperatures, 110°C and 560°C, of the drying and volatilization steps, respectively, were set slightly higher then 105°C and 550°C used for the VSS determinations because the actual temperatures in the TGA furnace tended to be a few degrees lower then the set–points. High precision in these temperatures was not considered a crucial factor. Negligible change in sample mass occurred after 15 min of drying at 110°C (samples had been pre–dried in air), therefore, a total drying time of 30 min, as opposed to 60 min, was dictated in order to reduce the time of the analysis. Determinations were based on the measured weight of the samples after 30 min at 550 °C. The temperature program was identical for all TGA determinations of the sample GAC, control GAC and biomass standard.

Advantage was taken of the weight suppression feature of the TGA balance controller during analysis of the GAC. A certain amount of the total sample weight can be pre–tared on the balance controller and the chart recorder can be adjusted so that the full–scale deflection represents a certain percentage loss of the total initial sample weight. This feature increases the precision in determining small fractional weight changes. For the control GAC, 95% of the initial sample weight was suppressed and at full–scale deflection the chart recorder indicated a 5% weight loss. A 90% weight repression was used for the sample GAC with a corresponding 10% weight loss at full–scale. Because the fractional weight loss was much larger, no weight suppression was used for the determinations involving the anaerobic biomass standard and the full–scale reading on the chart recorder was 10 mg.

7.2.3 SAMPLE PREPARATION

1. Sample GAC

The GAC from the bioreactor was washed several times in dd H_2O to

remove the residual medium and non–attached biomass. The GAC was then dried in a 105°C air oven for 48 h prior to the TGA determinations, primarily to remove moisture and adsorbed phenol.

2. Control GAC

One gram of 20–40 mesh, washed and dried F300 GAC, from the same stock that was initially charged to the bioreactor, was soaked for several weeks in a 25 mL solution of reduced medium (prepared as in Section 4.2) to which 60 mg of phenol had been added. The GAC was removed from this medium and prepared for the TGA determination by following the identical procedure as in preparation of the sample GAC.

3. Anaerobic Biomass Standard

Flocculated, anaerobic biomass removed from the bioreactors was used as a biomass standard because it was considered representative of the biomass attached to the GAC. A portion of the reactor tank contents containing suspended material was centrifuged (10000 rpm for 5 min), washed and re–suspended in dd H_2O. To ensure complete removal of the medium, this procedure was repeated twice more. The resulting biomass pellet was dried in an air oven at 105°C for 48 h (*i.e.* the same pre–treatment as the sample GAC received).

7.2.4 RESULTS

The results of the thermogravimetric analysis of the sample GAC and the control GAC are presented in Table 7.1. Net percent weight loss was defined as the percent weight loss after 30 min at 550°C minus the percent weight loss after 30 min at 105°C. Although the largest decrease occurred during ramping of the temperature from 105°C to 550°C, the weight of both the sample GAC and the control GAC continued to decrease at a steady–rate after 30 min at 550°C. The rate of decrease in both cases was identical and was attributed to slow pyrolysis of

TABLE 7.1: RESULTS OF THERMOGRAVIMETRIC ANALYSIS OF SAMPLE GAC AND CONTROL GAC

SAMPLE	FINAL WEIGHT[1] (mg)	% WEIGHT LOSS $\Delta \overline{m}_1$[2]	$\Delta \overline{m}_2$[3]	NET $\Delta \overline{m}$[4]
CONTROLS				
1	20.7	0.23	3.47	3.24
2	20.1	0.33	3.63	3.30
3	21.8	0.42	3.78	3.36
SAMPLES (FROM BIOREACTOR)				
1	28.6	0.81	8.36	7.55
2	26.7	0.48	8.52	8.04
3	27.3	0.43	8.18	7.75

Notes:

1. determined after 30 min at T_2

2. $\Delta \overline{m}_1$ = % weight loss from initial weight after 30 min at T_1 (110°C)

3. $\Delta \overline{m}_2$ = % weight loss from initial weight after 30 min at T_2 (560°C)

4. NET $\Delta \overline{m} = \Delta \overline{m}_2 - \Delta \overline{m}_1$

– all analyses performed under N_2 atmosphere

the carbon. Altering the time of the final mass measurement to 15 min at 550°C made little difference to the final results. Figure 7.7 is a sample of the chart recorder output of a typical TGA run on the sample GAC.

The TGA was performed on three samples of each type of GAC. The mean net percent weight losses were:

CONTROL GAC	3.30%	(s = 0.06)
SAMPLE GAC	7.78%	(s = 0.25)

The net percent weight loss of the sample GAC was attributed to loss of attached volatile solids, desorption of residual organics and a small degree of pyrolysis of the GAC. It was assumed that the weight loss due to the latter two effects was equal to entire weight loss of the control GAC. The amount of residual organics on the control GAC and the sample GAC was likely to be similar because both types of GAC received the same, long term (48 h) heating pre–treatment. The percent weight loss of the sample GAC which was attributed to loss of volatile solids was, therefore, determined by subtracting the net percent weight loss of the control GAC from the net percent weight loss of the sample GAC. The result was 4.48%.

The degree of biomass volatilization under a N_2 atmosphere at 550°C was expected to be quite different from what occurs under air at the same temperature. A factor to correct for this difference was calculated from the results of separate TGA determinations of the anaerobic biomass standard under N_2 and air (Table 7.2). TGA determinations of single samples only were possible because of a limitation on access to the necessary equipment. The very good reproducibility between TGA determinations of different GAC samples (Table 7.1) indicated that the method was reliable and that, although determinations of single samples were not ideal, they would provide reasonable estimates. A significantly larger fraction

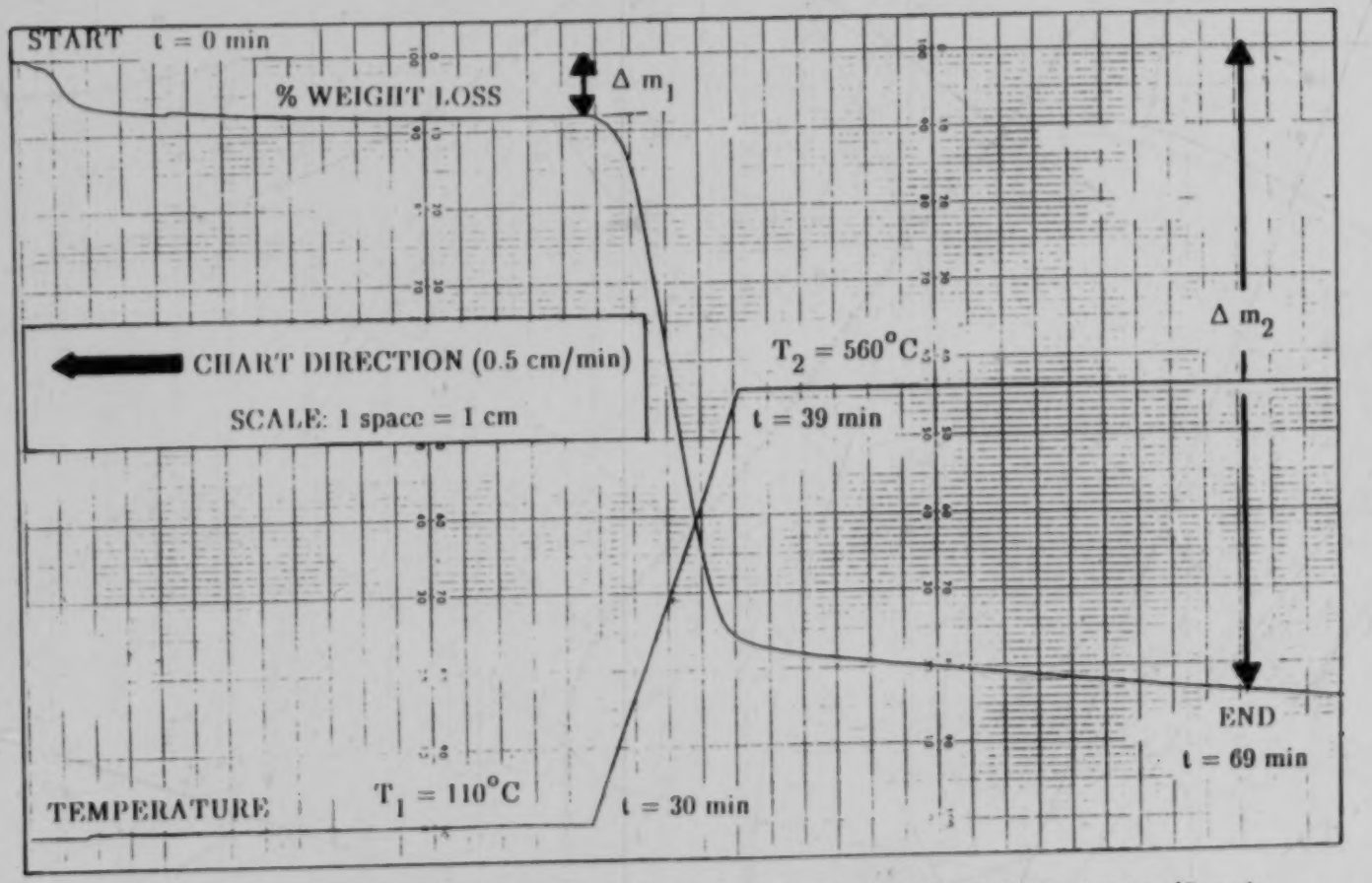


FIGURE 7.7: Reproduction of chart recorder output for a typical TGA determination (Sample GAC Run 1). Sample temperature and % weight loss are plotted on vertical scale. Full scale vertical deflection (from top to bottom) represents 10% loss of original sample weight. (Reproduction is 50% of original size)

TABLE 7.2: THERMOGRAVIMETRIC ANALYSIS OF ANAEROBIC BIOMASS STANDARD[1]

SAMPLE	ABSOLUTE WEIGHT (mg)		
	m_1[2]	m_2[3]	Δm[4]
UNDER N_2 ATMOSPHERE	7.74	4.26	3.48 (45.0 %)
UNDER AIR ATMOSPHERE	3.67	0.33	3.37 (91.8 %)

Notes:

1. Washed and dried flocculated biomass from bioreactor
2. m_1 = sample weight after 30 min at T_1 (110°C)
3. m_2 = sample weight after 30 min at T_2 (560°C)
4. $\Delta m = m_1 - m_2$

(91.8%) of the biomass was volatilized in air then in N_2 (45.0%). The correction factor was calculated to be:

$$CF = \frac{91.1\%\ VS\ in\ air}{45.0\%\ VS\ in\ N_2} = 2.04\ \frac{VS\ in\ air}{VS\ in\ N_2}$$

Again it was observed that the greatest loss in weight occurred during ramping of the temperature between 105°C and 550°C. After 30 min at 550°C under an air atmosphere, very little change in weight of the biomass sample was taking place, however, under an N_2 atmosphere the gradual decline in weight was observed. Using a final time of 15 min at 550° C the correction factor was calculated to be 2.10 compared to 2.04 at using 30 min. The determination of the correction factor was, therefore, only a slight function of the final time selected.

The final concentration of volatile solids on the GAC from the reactor was determined to be:

$$X_{b/c} = \frac{(4.48\%\ VS\ in\ N_2)}{(100\%)} \left[2.04\ \frac{VS\ in\ air}{VS\ in\ N_2}\right]$$

$$= 0.0914\ \frac{g\ VS\ in\ air}{g\ GAC}$$

This result is based on the initial GAC particle weight and, therefore, includes the mass of the attached biomass in the denominator. Converting to a dry, biomass free basis the results becomes:

$$x_{b/c} = \frac{X_b}{1 - X_b} = \frac{0.0914}{1 - 0.0919} = 0.101\ \frac{g\ VS\ in\ air}{g\ GAC}$$

This calculation assumes that the mass of residual adsorbed material and fixed solids that remained on the GAC after the pre–treatment was negligible with respect to the total mass. This result was incorporated into the kinetic analyses of Chapters 5 and 6.

7.2.5 ERROR ANALYSIS

An estimate of the uncertainty involved in the TGA estimation of VS concentration is appropriate. A confidence interval for the determination of the percent loss of volatile solids under an N_2 atmosphere can be estimated using the statistical equation for the estimation of the difference between two means (Shaeffer and M^{c}Cleave, 1984):

$$(\bar{x}_1 - \bar{x}_2) \pm t_{\alpha/2} S_p \left[1/n_1 + 1/n_2 \right]$$

$$S_p = \left[\frac{(n_1 - 1) s_1^2 + (n_2 - 1) s_2^2}{n_1 + n_2 - 2} \right]$$

$$df = n_1 + n_2 - 2 \qquad (7.1)$$

where:

$\bar{x}_1, \bar{x}_2$ are the respective means

s_1, s_2 are the respective standard deviations

n_1, n_2 are the respective sample sizes

df is the degrees of freedom

$t_{\alpha/2}$ is the Students t–distribution parameter

$1 - \alpha$ is the confidence interval.

This equation assumes normal distributions and equal, unknown population

variances. At 95% confidence ($\alpha = 0.05$) and with 3 degrees of freedom the value of $t_{0.025}$, taken from a table of t–distributions, is 2,776. Using equation 7.1 the 95 percent confidence interval on the difference between the net percent weight loss between the sample GAC and the control GAC is:

$$(7.78 - 3.30) \pm (2.776)(0.182)[1/3 + 1/3]^{1/2} = 4.48 \pm 0.41$$

or

$$4.48 \pm 9.2\%$$

A rigorous statistical analysis on the determination of the correction factor, CF, was not possible because determinations of only single samples were made. The degree of uncertainty of the CF was approximated by assuming the same relative standard deviation in the TGA determinations of the biomass standard as was observed in the triplicate determination for the sample GAC i.e. 3.2%. The relative error in the correction factor was then calculated to be:

$$\frac{\delta CF}{CF} = \frac{\delta(\text{NET } \Delta m \text{ in air})}{\text{NET } \Delta m \text{ in air}} + \frac{\delta(\text{NET } \Delta m \text{ in } N_2)}{\text{NET } \Delta m \text{ in } N_2}$$

$$= 3.2\% + 3.2\% = 6.4\%$$

The relative error on $X_{b/c}$ can be estimated from the sum of the relative errors of the percent loss of volatile solids ($4.48 \pm 9.2\%$) and of the correction factor

$$\frac{\delta X_{b/c}}{X_{b/c}} = 6.4\% + 9.2\% = 15.6\%$$

This relative error of the concentration of volatile solids on the GAC on a biomass free GAC basis, $x_{b/c}$, will be the same. An estimate of the range of uncertainty in the final result is, therefore:

$$x_{b/c} = 0.101 \frac{g \ VS \ in \ air}{g \ GAC} \pm 16\%$$

or, in absolute terms:

$$0.101 \pm 0.02 \frac{g \ VS \ in \ air}{g \ GAC}.$$

7.2.6 DISCUSSION

The major advantage of the TGA technique is that it provides a measure of biomass concentration on the GAC that can be directly compared to biomass determinations by the standard VSS technique. In fact, this is exactly what was done in the kinetic analyses of Chapters 5 and 6. Reproducibility between GAC samples and controls was very good (Table 7.1). Standard deviations for triplicate determinations of the sample GAC and control GAC were 1.8% and 3.2%, respectively. This reproducibility was attributed to the excellent sensitivity of the TGA equipment and the ability to carefully control test conditions. An additional advantage of the method, which is of some practical significance in experimental work and process monitoring, is that the required sample sizes are small. Less then 0.1 g of GAC sample was required for triplicate determinations.

One of the drawbacks of the method is that it relies on the availability of the TGA equipment. Another chief limitation is that it has not been verified or standardized. An attempt was made at using elemental analysis as a basis for verification of the TGA result. It was hoped that, with a biomass loading on the

order of 10%, the sensitivity of the elemental analysis would be sufficient to provide good estimates. Elemental analyses of the sample GAC, the control GAC, the anaerobic biomass standard (flocculated biomass from the bioreactor) and anaerobic biomass from the 2–L digester that served as an inoculum are summarized in Table 7.3. The analyses were performed in the Microanalytical Laboratory in the Department of Chemistry at the University of Alberta, Edmonton, Alberta. Results from the analysis of biomass from the reactor were not used because the proportion of carbon was unusually high. This was probably a result of contamination of the biomass with GAC fines. Based on separate mass balances on atomic C, N and H, the biomass concentration was determined to be 0.088 g VS/g GAC, 0.43 g VS/g GAC and 0.094 g VS/g GAC. Agreement between the results of the C and H balance is fairly good and the results tend to confirm the TGA result of 0.101 g VS/g GAC. The result from the N balance appears to be a large over–estimate and is due to a high content of the element N in the sample GAC. This is illustrative of the questionable reliability of elemental analysis in determining the attached biomass concentration.

Summarizing, the TGA technique shows promise as a method for measuring the concentration of biomass, as volatile solids, on the GAC in the anaerobic, phenol–degrading system and might serve equally well for similar determinations in other systems that employ carbonaceous carriers. It is limited as a measure of biological activity because, as with the standard VSS technique, it measures only bulk biomass and not active cells. Development of effective standardization and verification methods is necessary to prove the legitimacy and accuracy of the technique. A proposed method of standardization is to make TGA determinations on carefully prepared standards of biomass and GAC, the composition of which are accurately known.

TABLE 7.3: ELEMENTAL ANALYSIS OF GAC AND ANAEROBIC BIOMASS

SAMPLE	%C	%N	%H
CONTROL GAC[1]	87.1	0.61	0.54
SAMPLE GAC[2]	83.5	3.12	0.60
STANDARD BIOMASS[3]	64.1	6.13	4.28
2–L DIGESTER[4]	42.8	8.98	5.64

Notes:

1. clean GAC soaked in phenol–containing medium
2. colonized GAC from bioreactor
3. washed flocculated biomass from bioreactor
4. washed biomass from 2–L digester (bioreactor inoculum)

7.3 PHENOL ADSORPTION KINETICS

7.3.1 OBJECTIVE

The degree of both hydraulic shear and inter–particle abrasion, two mechanisms of removal of biomass from the GAC, are functions of the hydraulic condition of the bed. Inter–particle abrasion is minimal in fixed and expanded beds. Hydraulic shear and inter–particle abrasion are more severe in fluidized–bed operation. The preference of the bacteria for the sheltered area of the GAC surface, the macropores and crevices, was shown in the S.E.M. micrographs. Sparseness of growth on the exposed, outer surface of the GAC particles removed from the expanded bed reactor indicates that bacterial attachment is sensitive to the hydraulic conditions. The surface features of the GAC provide an environment where the bacteria are protected from the effects of hydraulic shear and inter–particle abrasion. The extent of pore colonization, therefore, was expected to be insensitive to bed hydraulic conditions.

This hypothesis was tested indirectly by measuring the effect of varying the hydraulic mode of the bed on the phenol adsorption kinetics. Kindzierski (1989) showed that the kinetics of phenol adsorption on GAC that had been exposed to anaerobic growth was significantly different then adsorption kinetics on clean GAC. Blockage of the macropores by dense bacterial growth was cited as the the cause of reduced phenol uptake rate and adsorption capacity. Similar, if not more extensive, blockage of the pores was observed in S.E.M. micrographs of the GAC removed from the reactor column. If biomass removal from the macropores occurred under the more severe hydraulic shear and inter–particle abrasion conditions of fully fluidized operation then the uptake rate and the adsorption capacity should increase. If, as the S.E.M. study suggests, the bacterial growth in the pores is insensitive to bed hydraulics then no difference in the adsorption kinetics should be

observed.

The adsorption kinetic measurements were a convenient means of determining the effect of bed hydraulics on biomass attachment because they could be performed *in situ*, without upsetting the anaerobic condition of the reactor. Direct studies, such as S.E.M., require sampling of the bed which was not easily done without risking exposure of the culture in the reactor to oxygen.

7.3.2 PROCEDURES

The adsorption kinetics experiments were performed during the trial period of the bioreactor operation. Two different hydraulic modes of the GAC were studied; the expanded–bed mode of normal operation and a fully fluidized mode. The phenol adsorption kinetics were first measured after the bed had been operating for some time in the expanded condition. After six days of continuous fluidization, the bed was once again returned to the initial expanded condition and the test was repeated. To determine the effect of bed mixing on the observed kinetics, a third determination was performed with the bed operating in fluidized mode during the test.

The first test was commenced after 165 d of bacterial growth on the GAC. The reactor was operated in batch mode throughout the experimental period. Feeding was suppressed for seven days to allow the concentration of phenol in the supernatant to drop to less than detectable levels. The recirculation pump was set at one 1.0 and provided a flowrate of 42.4 mL/min through the column at an upwards superficial velocity of 5.3 cm/min. The recirculation was momentarily stopped and 12 mL of a 30 200 mg/L reduced phenol feed solution was added to the tank. At time zero the recirculation was started. Samples (1–mL) were withdrawn from the tank every 5 min initially, every 10 min after 50 min and every 20 min after 80 min. Sampling was stopped after 3 h. Phenol concentration of the samples

was determined by the GC method of Section 4.4.

After completion of sampling, the recirculation pump was set at 6, providing a flowrate of 331 mL/min at a superficial velocity of 41 cm/min. At this flowrate, the GAC bed was fully and vigorously fluidized. After six days of continuous fluidization the bed was returned to the original expanded state and the adsorption kinetics determination was repeated as described above. The third determination was made six days later. Just prior to addition of the phenol, the recirculation pump was set at 6, to re–establish the same fluidized bed condition, and remained at this setting throughout the sampling period. Following the determination the bed was returned to the normal expanded mode.

7.3.3 RESULTS AND DISCUSSION

The results of the three determinations are presented in Figure 7.8. The period prior to each test, during which no phenol was fed to the reactor, was considered sufficient to return the carbon to its original state. Gas production ceased towards the end of the period before the test indicating that the phenol adsorbed onto the GAC had been desorbed and egraded. A certain amount of phenol may have be irreversibly bound to the GAC, lowering the capacity relative to fresh GAC, but it was assumed that this quantity remained fixed during the experiments.

An initial mixing time was estimated for each test by extrapolating the concentration versus time data to the time zero supernatant concentration, determined from the volume and concentration of the phenol addition. This mixing time, which was 2 min. and 3.5 min. in the first two tests, respectively, was subtracted from each sample time, effectively shifting the curves of Figure 7.8 to the left. The mixing time in the third test was negligible. The effect of phenol biodegradation over the 3–h time period was assumed to be small.

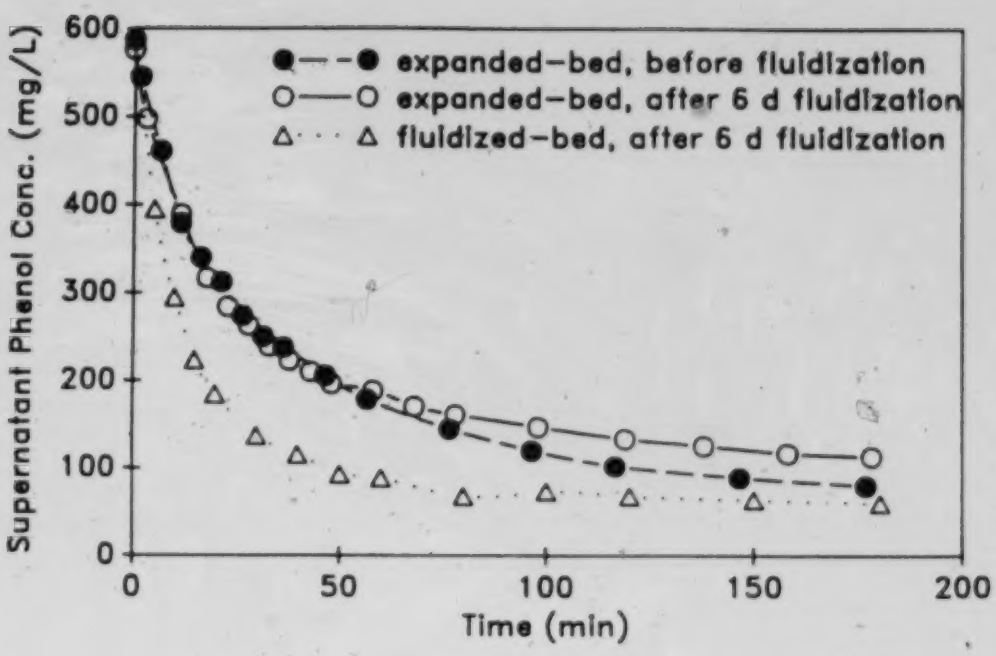


FIGURE 7.8: Kinetics of phenol adsorption onto GAC from bioreactor column determined *in situ*; concentration of phenol in the reactor supernatant versus time for 3 modes of GAC bed operation: (1) 12% expansion, before fluidization, (2) 12% expansion, after 6 d of continuous fluidization (3) fully fluidized, after 6 d of continuous fluidization.

The phenol adsorption kinetics were not significantly altered after six days of fluidization. In fact, the initial kinetics (at time < 50 min) are indistinguishable from the original kinetics and the longer term (at time > 50 min) uptake rate appears to be even lower. This qualitative observation, therefore, supports the hypothesis that the attached growth was insensitive to a change in hydraulic conditions between expanded and fluidized modes. This hypothesis was confirmed by the S.E.M. observations.

The noticeable increase in the rate of phenol adsorption while the bed was fully fluidized indicated that the observed kinetics in these experiments were a function of the bed mixing conditions. The Reynolds numbers for the expanded and fluidized conditions studied were well within the laminar range. Based on the superficial velocities and an average particle size of 620 μm, the Reynolds numbers were calculated to be 0.7 and 6 for the expanded and fluidized beds, respectively. Mass transfer limitations in the bulk fluid, rather then inside the GAC particles themselves, therefore, probably determined the adsorption rate. The effect of any change in intra–particle mass transfer limitations by virtue of biomass loss on the uptake rate may not have been observed.

A change in final adsorption capacity, however, should still be recognizable. Figure 7.8 suggests that the adsorption capacity after fluidization was not increased and, therefore, the extent of pore blockage was not reduced. The bottom curve on Figure 7.8 indicates that phenol uptake was almost complete after 100 min when the bed was fluidized. The curve for the expanded bed reactor was approaching the same equilibrium condition after 3 h. Because the initial conditions were the same for both tests, the adsorption capacity of the GAC was the same as well. A continuing small decrease in the phenol concentration at the end of the 3 h test period, resulting from bio–activity, was expected to be similar in each test and, therefore, it is reasonable to compare the curves of Figure 7.8 at large times.

The results of the adsorption kinetics experiments provide additional information that allows a comparison of the relative time scales of the adsorption and biological processes in the GAC cultures of the shock loading experiments. The half–life of phenol adsorption at fluidized bed conditions was estimated from Figure 7.8 to be about 12 min. The half–life of phenol degradation in the most active GAC serum bottle culture (culture 1, post–shock degradation, Figure 6.12) was about 40 h, or three orders of magnitude greater then the adsorption half–life. It can be assumed that adsorption of phenol onto the GAC quickly reached an equilibrium after the initial shock loadings of the GAC cultures and remained in equilibrium thereafter. Mass transfer gradients within the particle resulting from adsorption dynamics were, therefore, negligible and the local concentration of phenol in the vicinity of the particle surface was, therefore, the same as in the bulk liquid. This result is consistent with the conclusion of the shock loading experiments *i.e.* that mass transfer effects offered little, if any, protection of the GAC supported culture.

7.4 FLOC BED REACTOR

7.4.1 OBJECTIVE

The establishment of a stable layer of flocculated biomass above the GAC inspired the following question. Does the GAC play a role in the stabilization and retention of the biomass bed? If the GAC behaved in an inert manner with respect to the biomass layer, then a reactor design in which the activity was contained solely in a dense bed of flocculated biomass would be most logical. The GAC support would not be required and the proportion of the reactor volume occupied by inert carrier would be reduced, following the UASB design concept which was discussed in Chapter 3. In the treatment of an authentic phenolic waste,

removal of non biodegradable inhibitory compounds and the buffering of shock loads could be achieved in a separate GAC adsorption unit located upstream of the bioreactor. Replacement or regeneration of the GAC would not involve disturbance of the bioreactor and biomass removal.

To answer this question, an attempt was made at operating a reactor at steady–state in which the biomass was concentrated as a bed in an upflow column without the addition of GAC.

7.4.2 PROCEDURES

A second reactor was built and started–up concurrently with the bioreactor employed in the kinetic studies. Reactor construction and start–up procedures were identical to the first reactor. At start–up, the second reactor also contained GAC. As in the first reactor, a layer of biomass established above the GAC bed.

After 3 months of operation in batch and continuous modes, the support screen in the column of the second reactor clogged and slipped out of place. The flocculated biomass layer was then washed–out of the column into the tank by fluidization of the bed and the column was isolated and removed from the tank. After removal of the GAC, cleaning and replacement of the screen, the column was filled with reduced medium and reconnected to the tank anaerobically. This time, no GAC was added to the column.

Flocculated biomass from the tank was washed into the top of the column by reversing the recirculation flow temporarily. The recirculation pump was then set at 1, resulting in the same upward flow conditions as in the CFRB study, and the reactor was prepared for continuous operation.

7.4.3 RESULTS AND DISCUSSION

The attempt at establishing a stable layer of flocculated biomass failed.

Initially, the lighter biomass material washed–out of the column, but a bed of approximately 2 cm stabilized on the support screen. During this time the culture was starved of phenol and no gas was being produced. The reactor was given a batch feeding of phenol to about 200 mg/L. The bed was still stable 1 h following the feeding, however, after 16 h the entire biomass bed had been washed out of the column and into the tank.

The bed was again re–established in the column but once again was washed–out within 16 h of feeding. The procedure was repeated with the same results, total bed wash–out, even when the upward flowrate in the column was reduced by half. The proposed cause of the biomass wash–out was flotation of the floc particles. Gas produced from methanogenic phenol degradation was trapped within the biomass flocs causing them to become buoyant. They were then easily washed–out with the upwards flow of medium. Closer observation revealed that, within 12 h of feeding, most of the flocculated biomass had floated to the top of the column even though the recirculation flow had been stopped. Flotation of biomass is a problem commonly encountered in the start–up and operation of UASB reactors (Lettinga *et al.*, 1983).

These qualitative observations strongly suggest that the GAC performs a vital role in stabilizing the flocculated biomass layer above the bed. Under identical upflow velocities, the layer above the GAC bed stabilized and slowly grew while in the reactor without GAC the layer of flocculated biomass, that had been previously stabilized above a GAC bed, was completely washed–out. A mechanism was proposed to explain the stabilization effect of the GAC. Large gas bubbles are produced and trapped within the GAC bed. After reaching a certain size and pressure, the bubbles are released and travel upwards through the bed. In doing so, they gently agitate the flocculated biomass layer. This agitation serves to release gas bubbles produced within the flocs themselves, which are entrained by the larger

bubbles rising from the GAC bed. The GAC bed and the flocculated biomass layer then settle to their original state. Visual observation of the GAC reactor column revealed the formation of large bubbles within the GAC bed. Their periodic release and the resulting gentle agitation of the biomass layer could be viewed through the glass wall of the column. If this release of bubbles from the GAC bed is frequent enough, the amount of gas retained in the flocs will never reach the level which causes flotation. This mechanism was presumed to account for the excellent retention of the flocculated biomass layer above the GAC bed.

8 SUMMARY AND DISCUSSION

8.1 ACHIEVEMENT OF OBJECTIVES

The overall objective of this research project was to establish the mechanisms which account for the superior performance of granular activated carbon as a biological support medium in the anaerobic bio–treatment of phenolic wastewaters. Previous work had established the effectiveness of GAC in improving the treatment of authentic wastewaters by adsorbing inhibitory compounds and buffering the system against surges in organic loadings. Further enhancement of system performance by more subtle mechanisms was suspected. Optimization of the design of treatment processes requires an understanding of these mechanisms. Despite the fact that some of the expected modes of enhancement that were investigated were not significant, the experimental studies provided some new insights into the benefits of GAC as a biological support.

The near steady–state kinetic study showed that the phenol–degrading activity of a culture supported on GAC particles in an expanded bed was not greater than that of un–supported cultures. The activity of the supported, flocculated and suspended phenol–degrading cultures were, given a reasonable range of experimental error, quite similar. The shock loading experiments showed that attachment to GAC offered no protection of the culture from the toxic effects of phenol on the phenol–oxidizing organisms at high supernatant concentrations (> 1500 mg/L). The time scale of phenol adsorption was more than an order of magnitude smaller than that of biological degradation, therefore, no diffusional gradients existed that might result in local concentrations that were more favorable to the organisms. Thermogravimetric analysis showed quantitatively that the concentration of dry biomass on the GAC particles from the expanded bed reactor was only about 10% by weight, even after more then 200 d of continuous growth.

S.E.M. showed that growth was still largely concentrated in the surface pores and crevices of the GAC and that no continuous biofilm was formed.

These results, considered alone, suggest that employing GAC as a support medium is not a favorable method of increasing biomass retention in this system. The proportion of active biomass to carrier is low and, combined with a lack of kinetic enhancement, results in a low volumetric degradation activity. Other than simple adsorption, there are no protective mechanisms offered by the GAC which would serve to increase process stability. From this standpoint, overall treatment efficiency would be improved by removal of inhibitory compounds and buffering in a separate GAC adsorption unit located upstream of the biological treatment reactor. More efficient biomass retention in the bio–reactor could be achieved by establishment of a sludge bed (i.e a UASB reactor) or utilization of a more efficient support medium. Kindzierski's (1989) study indicated that Ambergard XE–352 anionic exchange resin was able to retain a higher concentration of active biomass than GAC under identical culture conditions.

The observation of a stable layer of settled biomass which rapidly developed above the expanded GAC bed, and contributed significantly to the overall phenol–degrading activity of the reactor, suggested some unexpected mechanisms by which GAC could serve to improve the efficiency of a treatment process. Most important was the role of GAC in promoting the stabilization and retention of this sludge bed. The failed attempt at establishing a similar reactor without the GAC bed suggested a mechanism of sludge bed stabilization in which flotation of the flocculated biomass was prevented by the methanogenic activity of the culture supported on the GAC. The effective density of the GAC particles, the quantity of gas produced within the GAC bed and the hydraulic regime combined to provide ideal conditions for retention of the sludge bed.

Rapid development of the sludge bed was encouraged by re–seeding of the

supernatant liquid with active biomass by the GAC. The fact that a complete biofilm could not be supported meant that further growth on the GAC was transferred into the liquid and accumulated in the sludge bed. S.E.M. micrographs of biomass particles removed from the sludge bed indicated the onset of granulation, although visual observation of the reactor showed that much of the biomass material was composed of lighter, flocculated particles. In principle, granulation is the most efficient method of retaining biomass because no inert carrier is required. The incorporation of what were deduced to be activated carbon fines into the matrix of the granules was observed. These fines increased the effective density of the granules, improving their settling properties, and may also have served as a point of nucleation in granule formation. Although the morphologies appeared different, S.E.M. studies indicated some physical similarities (*i.e.* size, matrix of extracellular polymer) between the granular particles and the colonies immobilized within the larger pores of the GAC. This fostered speculation that the macropores of the GAC may serve as an ideal environment for the initial formation of granules that are later released into the liquid as part of the re–seeding process. The adsorption kinetics studies, combined with S.E.M. observations of the supported colonies, showed that the surface features of the GAC provide an environment where the culture is protected from the effects of shear and inter–particle abrasion. The unique surface geometry of the GAC, therefore, may serve to promote granulation in addition to re–seeding the supernatant.

The superior response of the GAC–supported culture to lower levels of phenol shock loading (< 1400 mg/L) was attributed to the GAC adsorption capacity. It was proposed that the concentration of the pathway intermediate, acetic acid, was reduced to levels low enough to prevent feedback inhibition of phenol degradation by adsorption to the GAC. This implies that GAC employed *in situ* in anaerobic treatment reactors may improve the stability of the system by

adsorbing small amounts of inhibitory pathway intermediates that are produced during process upsets. Further work is necessary to substantiate this hypothesis.

8.2 IMPLICATIONS FOR TREATMENT REACTOR DESIGN

The rapid development and stabilization of a bed of active biomass above the expanded bed of GAC in the bioreactor is significant and could lead to new concepts in the design of treatment reactors. Retention of biomass as a bed of flocs or granules is a far more efficient utilization of reactor volume than immobilization on a solid support. In the bioreactor, the portion of the bed volume occupied by the biomass layer was about 40%, yet this biomass accounted for approximately 66% of the phenol–degrading activity of the reactor. New growth on the GAC was being transferred into the biomass bed and, combined with the growth of the flocs and granules themselves, was resulting in a continuous increase in this ratio in favor of the sludge bed. The high system dilution rate resulted in the rapid wash–out of suspended biomass and applied a selection pressure for the settling biomass particles.

In terms of design of treatment reactors, concentration of biomass in a sludge bed is more favorable than attachment to GAC because of the greater biomass density. In the expanded–bed reactor, the GAC was vital to the retention of the sludge bed and may have encouraged granule formation. These observations imply that a hybrid reactor design, that combines the basic elements of the upflow anaerobic sludge blanket (UASB) process with an expanded bed of GAC, would have advantages over existing technologies. In this dual–bed design, biomass would be concentrated in a sludge bed located above a bed of GAC in an upflow column. Expanded operation of the GAC bed would prevent channeling and plugging and would ensure even mixing within the reactor. Expansion of the GAC would most likely necessitate recycling of the medium. Retention of biomass could be enhanced

further by a gas separator and baffle system similar to those installed in UASB reactors.

The GAC would serve multiple purposes in the proposed reactor design. Removal of toxic and inhibitory compounds and buffering against the effects of surges in organic loading would still be provided for by the adsorption capacity of the GAC. In addition, the GAC would help to develop and stabilize a layer of flocculated and granular biomass as well as serving as a support for active biomass.

The volumetric efficiency of this hybrid reactor would be much higher than in conventional expanded or fluidized–bed anaerobic bioreactors because of the greater biomass density of the sludge bed. The hydraulic stabilization feature of the GAC bed would reduce the minimum size of the biomass particles that can be retained. Retention of small particles, that would otherwise wash out of a UASB reactor at the same upward flow velocity, would reduce the time required for a significant size sludge bed to form during reactor start–up. The success of UASB reactor operation is dependent on the formation of large biomass granules. This limitation would not be as severe in the proposed design. Gentle agitation by bubbles rising from the GAC is ideally suited to the task of providing bed mixing and degassing of the sludge bed. Mechanical mixing would not be required.

Hydraulic stablization of a sludge bed could also be achieved by alterante means. Gentle bubbling of an inert gas, such as nitrogen, upwards through the sludge bed might achieve the same stabilization effect as the GAC bed. This system would not benefit from the re–seeding capability of the GAC culture, which would be very useful in the initial development of a stable sludge bed during reactor start–up. It would also represent an additional capital and operating cost to the process. However, such a system might be very useful in studying sludge bed hydrodynamics in an experimental reactor.

9 CONCLUSIONS

1. No enhancement of the phenol–degrading activity of an anaerobic culture by virtue of immobilization on GAC was observed. Specific activities of the GAC supported, flocculated and suspended cultures were 0.14–0.19 mg phenol/mg VS/·d.

2. Re–seeding of the supernatant liquid with active culture by the colonized GAC promoted the development of a significant sludge bed above an expanded bed of GAC. Gas production within the GAC bed enabled retention of the sludge bed. This phenomenon has important implications in the design of treatment reactors.

3. The onset of granulation was detected in the anaerobic phenol–degrading system. The unique features of the GAC may be conducive to granule formation.

4. No complete biofilm was developed on the GAC particles in the anaerobic expanded–bed reactor. Growth was concentrated in the surface macropores and crevices of the GAC and was not affected by the hydraulic mode of bed operation. The concentration of biomass on the GAC, estimated by thermogravimetric analysis, was 0.101 ± 0.02 g VS/g GAC.

5. Adsorption to GAC may have served to reduce the effects of system stresses that resulted in the accumulation of acetic acid, the metabolic pathway intermediate of phenol degradation, to inhibitory levels.

6. Immobilization on GAC did not protect cultures from the effects of shock loads of toxic phenol concentrations. Diffusional limitations were not sufficient to result in a local phenol concentration on the GAC surface that was significantly

lower than in the bulk medium.

10 RECOMMENDATIONS

Thermogravimetric analysis proved a promising technique for measurement of biomass attached to the GAC in the anaerobic phenol–degrading system. The method requires verification for it to become a generally accepted technique for this type of measurment. Verification would entail TGA determinations of several carefully prepared standards of which the biomass composition was known *a priori*.

2. The observation of granule formation in the phenol–degrading system was significant. The factors involved in initiating granule formation are not yet fully understood. In particular, the role of activated carbon in promoting granule formation should be investigated, including a more detailed study of the granules themselves (elemental analysis, inorganic content, sectioning and S.E.M) and laboratory or pilot–scale performance tests. The effect of the addition of activated carbon fines on granulation should be evaluated.

3. The shock loading experiments provided some insights into the mechanisms of inhibition of phenol degradation and the possible role of GAC in reducing the effect of inhibition by intermediates, however, the data collected were not sufficient to draw positive conclusions. A series of shock loading experiments similar to those performed in this study but with accurate monitoring of acetic acid concentration and methane production would verify this mechanism. The apparent indifference of the methanogenic bacteria to completely inhibitory concentrations of phenol in the most severely stressed cultures could be verified by addition of acetic acid and H_2, as substrates for the methanogens, following inhibition of the phenol–oxidizers by the shock load. The hypothesis of adsorption of acetic acid on the GAC may be directly verified by determination of an adsorption isotherm.

4. The validity of the conclusion of incomplete biofilm formation on the GAC, which was based on S.E.M. observation, should be verified by determining the exten of biofilm removal which might occur as a result of the S.E.M. sample preparation procedure. Radio–labelled cell studies may achieve this. If removal is significant, the sample preparation procedure should be modified to prevent bioflim loss.

5. Development of a rational design basis for a dual–bed reactor should start with design and construction of laboratory or pilot–scale reactors. The effect of various loadings of GAC, hydraulic conditions and bed geometries on the retention of the sludge bed should be investigated. An efficient start–up procedure, which results in the shortest start–up period, should be devised to make the process attractive.

REFERENCES

APHA. 1985. Standard methods for the examination of water and wastes. 16th ed. American Public Health Association, American Water Works Association and Water Pollution Control Federation. Washington, D.C. 1268 pp.

Bailey, J.E. and D.F. Ollis. 1986. Biochemical Engineering Fundamentals. 2nd ed. McGraw–Hill, New York. 984 pp

Chang, H.T. and B.E. Rittmann. 1986. Biofilm loss during sample preparation for scanning electron microscopy. Water Res. 20: 1451–1456.

Chen, S.J., T.L. Chun and W.K. Shieh. 1988. Anaerobic fluidized bed treatment of an industrial wastewater. J. Water Pollut. Control Fed. 60: 1826–1832.

Cohen, A., R.J. Zeotmeyer and A. van Deursen. 1979. Anaerobic digestion of glucose with separated acid production and methane formation. Water Res. 13: 571–580.

Costerton, J.W., Geesey, S.W. and K.J. Cheng. 1978. How bacteria stick. Sci. Amer. 238: 86–995.

Cross, W.H., E.S.K. Chan, S. Pohland, S.R. Harper, S. Kharkar and F. Lui. 1982. Anaerobic biological treatment of coal gasifier effluents. Biotechnol. Bioeng. Symp. No. 12. J. Wiley and Sons, New York, pp: 349–363.

Dolfing, J. 1985. Kinetics of methane fermentation by granular sludge at low substrate concentration: The influence of mass transfer limitations. Appl. Microbiol. Biotechnol. 22: 77–81.

Dolfing, J., A. Griffioen, A.R.W. van Neerven and L.P.T.M. Zevenhuizen. 1985. Chemical and bacteriological characteristics of granular methanogenic sludge. Can. J. Microbiol. 31: 744–750.

Doran, P.M. and J.E. Bailey. 1986. Effects of immobilization on growth, fermentation properties and macromolecular composition of *Saccharomyces cerivisiae* attached to gelatin. Biotechnol. Bioeng. 28: 73–87.

Dwyer, D.F., M.L. Krumme, S.A. Boyd, J.M. Tiedje. 1986. Kinetics of phenol biodegradation by an immobilized methanogenic consortium. Appl. Environ. Microbiol. 52: 345–351.

Evans, C.W. 1977. Biochemistry of the bacterial catabolism of aromatic compounds in anaerobic environments. Nature 270: 17–22

Fedorak, P.M. and S.E. Hrudey. 1983. A simple appparatus for measuring gas production by methanogenic cultures in serum bottles. Environ. Tech. Lett. 4: 425–432.

Fedorak, P.M. and S.E. Hrudey. 1984a. Batch anaerobic methanogenesis of phenolic coal conversion wastewater. Water Sci. Tech. 17: 143–145.

Fedorak, P.M. and S.E. Hrudey. 1984b. The effects of phenol and some alkyl phenolics on batch anaerobic methanogenesis. Water Res. 18: 361–367.

Fedorak, P.M., E. Knettig and S.E. Hrudey. 1985. The effects of activated carbon on the methanogenic degradation of phenolics in H–coal wastewater. Environ. Tech. Lett. 6: 181–188.

Fedorak, P.M.and S.E. Hrudey. 1986. Nutrient requirements for the methanogenic degradation of phenol and *p*–cresol in anaerobic draw and feed cultures. Water Res. 20: 929–933.

Fedorak, P.M. and S.E. Hrudey. 1987. Inhibition of anaerobic degradation of phenolics and methanogenesis by coal coking wastewater. Water Sci. Tech. 19: 219–228.

Fox, P., M.T. Suidan and J.T. Pfeffer. 1988. Anaerobic treatment of a biologically inhibitory wastewater. J. Water Pollut. Control Fed. 60: 86–92.

Gardner, D.A., M.T. Suidan and H.A. Kobayashi. 1988. Role of GAC surface activity and particle size during fluidized–bed anaerobic treatment of refinery sour water stripper bottoms. J. Water Pollut. Control Fed. 60: 505–513.

Harper, S.R., W.H. Cross, F.G. Pohland and E.S.K. Chian. 1983. Adsorption–enhanced biogasification of coal conversion wastewater. Biotechnol. Bioeng. Symp. No. 13. J. Wiley and Sons, New York. pp: 401–420.

Healy, J.B. and L.Y. Young. 1978. Catechol and phenol degradation by a methanogenic population of bacteria. Appl. Environ. Microbiol. 35: 216–218.

Henze, M. and P. Harremoes. 1983. Anaerobic treatment of wastewaters in fixed film–reactors – a literature review. Water Sci. Tech. 15: 1–99.

Hulshoff Pol, L.W., W.J. de Zeeuw, C.T.M. Velzeboer and G. Lettinga. 1983. Granulation in UASB Reactors. Water Sci. Tech. 15: 291–304.

Hungate, R.E. 1969. A roll tube method for cultivation of strict anaerobes. In: Methods in Microbiology, 3B. Ed: J.R. Norris and D.W. Ribbons, Academic Press. New York. pp 117–132.

Khan, A.K., M.T. Suidan and W.H. Cross. 1981. Anaerobic activated carbon filter for the treatment of phenol–bearing wastewater. J. Water Pollut. Control Fed. 53: 1519–1532.

Khan, A.K., M.T. Suidan and W.H. Cross. 1982. Role of surface active media in anaerobic filters. J. Environ. Eng. Div. Am. Soc. Civ. Eng. 108: 269–285.

Kindzierski, W.B. 1989. Enhanced anaerobic treatment of phenolic wastewaters. Ph.D. Thesis. Dept. of Civil Engineering, University of Alberta, Edmonton, Alberta. 383 pp.

Kim B.R., E.S.K. Chian, W.H. Cross and S.S. Cheng. 1986. Adsorption, desorption and bioregeneration in an anaerobic granular activated carbon filter. J. Water Pollut. Control Fed. 58: 35–40.

Knoll, G. and J. Winter. 1987. Anaerobic degradation of phenol in sewage sludge. Appl. Microbiol. Biotech. 25: 384–391.

Knoll, G. and J. Winter. 1989. Degradation of phenol via carboxylation to benzoate by a defined, obligate, syntrophic consortium. Appl. Microbiol. Biotechnol. 30: 318–324.

Kobayashi, T., T. Hashinaga, E. Mikani. and T. Suzuki. 1989. Methanogenic degradation of phenol and benzoate in acclimated sludges. Water Sci. Tech. 21: 55–65.

Lettinga, G., A.F.M. van Velsen, S.W. Hobma, W. de Zeeuw and A. Klapwizk. 1980. Use of the upflow sludge blanket (USB) reactor concept for biological wastewater treatment especially for anaerobic treatment. Biotechnol. Bioeng. 22: 699–734.

Lettinga G., L.W. Hulshoff Pol., W. Wiegant, W. de Zeeuw, S.W. Hobma, P. Grin, R. Roecsina, S. Sayed. and A.F.M. van Velson. 1983. Upflow sludge blanket process. Proc. 3rd Int. Symp. on Anaerobic Digestion. Boston, Mass. pp: 139–158.

Lin, C.Y., T. Noike, K. Sato and J. Matsumoto. 1987. Temperature characteristics of the methanogenesis process in anaerobic digestion. Water Sci. Tech. 19: 299–310.

Lyman, W.J., 1978. Applicability of carbon adsorption to the treatment of hazardous industrial wastes. In: Carbon Adsorption Handbook. Ed: P.N. Cheremisinoff and F. Ellerbusch. Ann Arbor Science, Mich. pp: 131–136.

Metcalf and Eddy. 1979. Wastewater Engineering: Treatment/Disposal/Reuse. 2nd ed. Metacalf and Eddy, Inc. McGraw–Hill Book Co., New York. 920 pp.

McCarty, P.L. and D.P. Smith. 1986. Anaerobic Wastewater Treatment. Environ. Sci. Technol. 20: 1200–1206.

Neufeld, R.D., J.D Mack and J.P. Strakey. 1980. Anaerobic phenol biokinetics. J. Water Pollutt. Control Fed. 52: 2367–2377.

Obayashi, A.W. and J.M. Gorgan. 1985. Management of Industrial Pollutants by Anaerobic Processes. Lewis Publishers, Inc., Mich. 193 pp.

Pfeffer, J.T. and M.T. Suidan. 1989. Continuous processing of toxic organics in a fluidized–bed GAC reactor employing carbon replacement. Biotechnol. Bioeng. 33: 139–148.

Sahm, H. 1984. Anaerobic wastewater treatment. In: Advances in Biochemical Engineering Technology. No. 29 Ed: A. Fiechter. Springer–Verlag, New York. pp 183–184.

Senthilnathan, P.R. and J.J. Ganzarczyk. 1988. Determination of biomass in activated sludge containing large quantities of carbonaceous carriers. Water Pollut. Res. J. Can. 23: 279–291.

Shaeffer, R.L. and J.T. McCleave. 1982. Statistics for Engineers. Duxbury Press, Boston. 475 pp.

Stanier, R.Y., E.A. Adelberg and J.L. Ingraham. 1976. The Microbial World. 4th ed. Prentice Hall, N.J.. 871 pp

Stronach, S.M. T. Rudd and J.N. Lester. 1986. Anaerobic Digestion Processes in Industrial Wastewater Treatment. Springer–Verlag, New York. 184 pp.

Suidan, M.T., W.H. Cross, M. Fong and J.W. Calvert. 1981. Anaerobic carbon filter for the degradation of phenols. J. Environ. Eng. Div. Am. Soc. Civ. Eng. 107: 563–579.

Suidan, M.T., G.L. Siekerka, S.W. Kao and J.T. Pfeffer. 1983a. Anaerobic filters for the treatment of coal gasification wastewaters. Biotechnol. Bioeng. 25: 1581–1596.

Suidan, M.T., C.E. Strubber, S.W. Kao and J.T. Pfeffer. 1983b. Treatment of coal gasification wastewater with anaerobic filter technology. J. Water Pollut. Control Fed. 55: 1263–1270.

Suidan, M.T., P. Fox and J.T. Pfeffer. 1987. Anaerobic treatment of coal gasification wastewater. Water Sci. Tech. 19: 229–236.

Switzenbaum, M.S. 1983. A comparison of the anaerobic filter and the anaerobic expanded/fluidized–bed process. Water Sci. Tech. 15: 345–358.

Taylor, G.T., 1982. The methanogens. In: Progress in Industrial Microbiology. No. 15. Ed: M. J. Bull. Elsevier Sci. Publ. Co., New York, pp 231–270.

van den Berg, L. 1984. Developments in methanogenesis from industrial wastewater. Can. J. Microbiol. 30: 975–990.

Wang, Y.T., M.T. Suidan and B.E. Rittman. 1986. Anaerobic treatment of phenol by an expanded–bed reactor. J. Water Pollut. Control Fed. 58: 227–233.

Wang, Y.T., M.T. Suidan, J.T. Pfeffer and I. Najain. 1989. The effect of concentration of phenols on their batch methanogenesis. Biotechnol. Bioeng. 33: 1353–1357.

Wiegant, W.M. and A.W.A. de Man. 1986. Granulation of biomass in thermophilic upflow anaerobic sludge blanket reactors treating acidified wastewaters. Biotechnol. Bioeng. 28: 718–729.

Wilkie, A. and E. Colleràn. 1988. Microbial aspects of anaerobic digestion. In: Anaerobic Treatment of Industrial Wastewaters. Ed: M.F. Trophy. Noyes Data Corp, Park Ridge, N.J. pp 35–48.

Yang, S.T. and M.R. Okos. 1987. Kinetic study and mathematical modeling of methanogenesis of acetate using pure culture methanogens. Biotechnol. Bioeng. 30: 661–667.

Young, J.C. and P.L. McCarty. 1969. The anaerobic filter for waste treatment. J. Water Pollut. Control. Fed. 41: R160.

Young, J.C. and M.F. Dahab. 1983. Effect of media design on the performance of fixed–bed anerobic filters. Water Sci. Tech. 15: 369–383.

APPENDIX I BIOREACTORS: EQUIPMENT

The bioreactor used in this study consisted of the following major components:

1. Well–mixed tank
2. Column and recirculation system
3. Feed system
4. Effluent collection and measurement system
5. Gas collection and measurement system

Except for some glassware, the system was assembled from standard components available from laboratory equipment suppliers. The glassware components of the the tank and column were manufactured by the glass blowing shop in the Department of Chemistry at the University of Alberta. All glass tubing employed was laboratory standard glass tubing, 6 mm O.D. x 1 mm wall size. Flexible tubing, for transfer of liquid medium or gas, was either Tygon surgical plastic tubing (5/16 inch O.D. x 1/16 inch wall) or butyl rubber tubing (1/2 inch I.D. x 1/16 inch wall). These materials were selected for their low oxygen permeability and biological inertness. Butyl rubber tubing was preferred for liquid transfer because of the lower oxygen permeability. A small amount of silicon grease was applied to all connections and joints to prevent leaks. All Tygon tubing connections were secured with two plastic cable ties and butyl rubber connections were secured with screw–type hose clamps.

I.1 TANK

The tank served as a reservoir for receiving feed and to provide mixing of the reactor contents. It also served as a gas–liquid separator, an effluent flow controller and a convenient location for liquid sampling. The tank was a cylindrical vessel

with a flat bottom and an open top as illustrated in Figure I.1. The vessel had dimensions of 9.0 cm I.D. x 15.0 cm total height and was constructed of 3.5 mm thick glass. A 0.5 cm x 0.5 cm lip was formed to the upper rim. The liquid level in the tank was controlled by an overflow exit consisting of a 1.0 cm O.D x 0.5 cm I.D. x 3 cm long glass tubular connector fused to the side of the tank at a height of 7.5 cm from the base and at a downwards angle of 45°.

The lid of the tank was a 38 mm thick butyl rubber stopper (#15) through which five holes had been bored to accommodate lengths of glass tubing. The five lengths of tubing inserted vertically through the stopper were for feed, gas, sample and two recirculation line connections. The lower ends of the feed and gas tubes were inserted into the headspace of the tank The sample and recirculation tubes were longer and extended below the liquid level to within 3 cm of the bottom of the tank. The rubber stopper was held in place in the top of the tank by an arrangement of large plastic cable ties fitting over the top of the stopper and under the lip on the tank rim.

Mixing was provided by a magnetic stirrer (Nova II Stirrer by Thermolyne) and a 4 cm magnetic stir bar. The tank was secured to the top of stirrer plate by a ring clamp and stand. The stirrer speed control was set at 6 during normal operation of the reactor to provide well mixed–conditions. At this speed the liquid hold–up in the reactor was determined to be 461 mL by direct measurement. With the rubber stopper inserted, the resulting headspace volume was 315 mL.

I.2 COLUMN AND RECIRCULATION SYSTEM

The GAC bed was retained in the vertical glass column depicted in Figure I.2. The column was constructed from a 14 cm long cylindrical piece of glass (3.2 cm I.D. x 2.5 mm wall). The top end of the column was open and the rim was flared. A 1.0 cm O.D. x 0.5 cm I.D. x 4.5 cm long tubular hose fitting was fused to

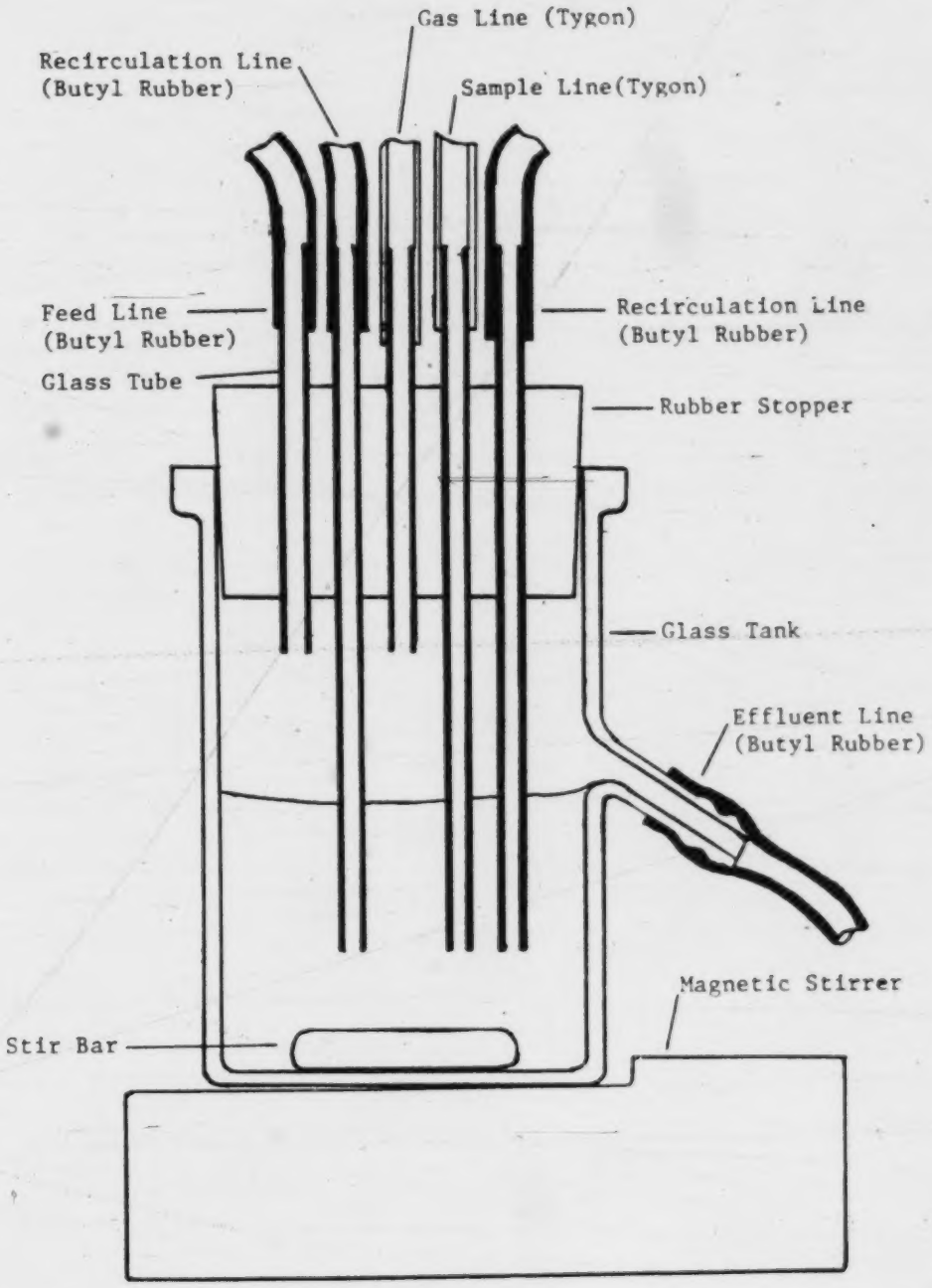


FIGURE I.1: Reactor Tank and Fittings

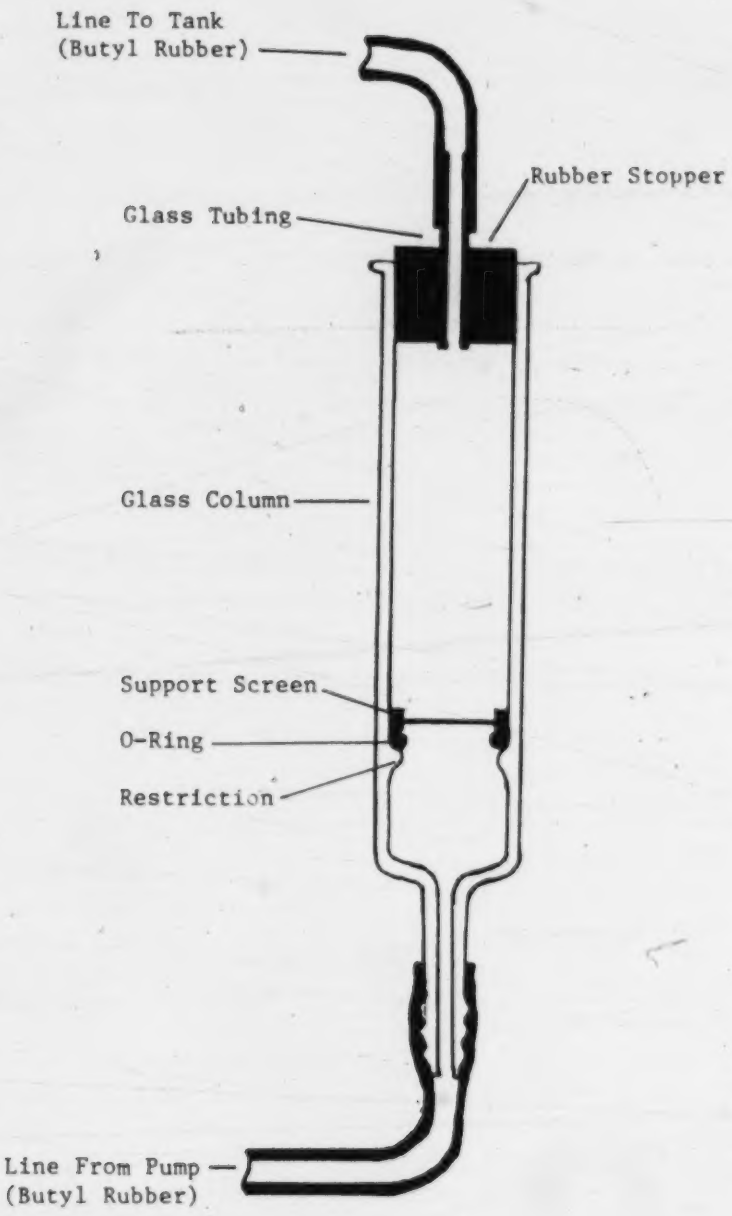


FIGURE I.2: Reactor Column

the bottom of the column. The activated carbon bed was supported on a screen constructed of 100 U.S. standard size stainless–steel mesh crimped to a 2.7 cm O.D. stainless–steel ring. A 3 mm restriction on the inside diameter of the column was formed by the blowing process 2 cm from the column bottom and supported the screen. A 2.8 cm x 2.2 cm rubber O–ring was placed between the screen and the restriction to prevent slipping of the screen. The O–ring and screen were secured by a small amount of epoxy glue. The top end of the column was sealed by a 21 mm deep (#6) rubber stopper. The exit from the top of the column was provide by a short length of glass tubing fitted through a hole in the stopper. The lower end of this tubing was flush with the stopper to prevent trapping of gas within the column. With the rubber stopper held firmly in place in the top of the column by plastic cable ties, the effective height above the screen was 9.4 cm. The empty–bed liquid hold–up in the column, including the fittings, was determined to be 84 mL by direct measurement.

Recirculation of medium was provided by a variable speed Masterflex Tubing Pump by Cole–Palmer. The pump controller/drive unit (cat # J–7520–25) operated between 6 and 600 rpm and featured a fine speed control. A no. 16 standard polycarbonate pump head with a stainless steel rotor was used (cat. # J–7016–25) in conjunction with a 75 cm length of no. 16 size Norprene rubber tubing (3.1 mm I.D.). This tubing combined a very long service life with low oxygen permeability. The range of flowrates provided by this arrangement were between 4.8 and 480 mL/min enabling a full range of bed operation from completely packed to fully fluidized. The direction of flow was reversible. During normal reactor operation the pump was removing liquid medium from the tank and supplying it to the bottom of the column. The medium was circulated through butyl rubber tubing. Three continuous sections were required to complete the recirculation loop. The lengths required were 100 cm between the tank and the

pump, 50 cm between the pump and the bottom of the column and 90 cm from the top of the column to the tank. The rubber tubing fitted directly to the glass tubes protruding from the top of the tank and the column and to the tubular fitting at the bottom of the column. Two 12 cm lengths of 1/4 inch O.D. x 1/32 inch O.D. wall stainless steel tubing were used to join the pump tubing to the butyl rubber tubing at the inlet and outlet of the recirculation pump (see Figure I.3). Based on the diameter and lengths of the tubing used, the liquid hold–up in the recirculation loop tubing was calculated to be 90 mL. The hold–up of the entire recirculation system, minus the GAC, was 174 mL.

I.3 FEED SYSTEM

The reactor feed system is shown in Figure I.4. Feed was supplied to the reactor tank by a Masterflex Tubing Pump (Cole–Palmer, cat # J–7567–20) with a no. 13 standard pump head (polycarbonate, stainless steel rotors, cat # J–7016–21) and no. 13 Norprene rubber tubing (0.8 mm I.D.). The variable speed pump motor operated between 1 and 100 rpm and was capable of delivering between 0.06 and 6 mL/min with the no. 13 size tubing. The pump tubing was connected to butyl rubber tubing (1/4 inch I.D. x 1/8 inch wall) using 8 cm lengths of 1/4 inch O.D. x 1/32 inch wall and 1/16 inch x 1/32 inch wall stainless steel tubing joined by 1/4 inch x 1/16 inch stainless steel swagelock adapters as indicated in Figure I.4.

A 4–L Erlenmeyer flask served as the feed reservoir. The flask was sealed at the top by a rubber stopper through which two lengths of glass tubing were inserted. The pump drew feed through a 50–cm length of glass tube which reached to within 0.5 cm from the bottom of the flask. The butyl rubber tube fitted directly to the top end of this piece. A 10 cm length of glass tube extended only into the headspace of the flask and was connected to two short lengths of 5/16 inch I.D. x 1/16 inch wall Tygon plastic tubing and 1/4 inch Nalgene Y–type connector as shown in

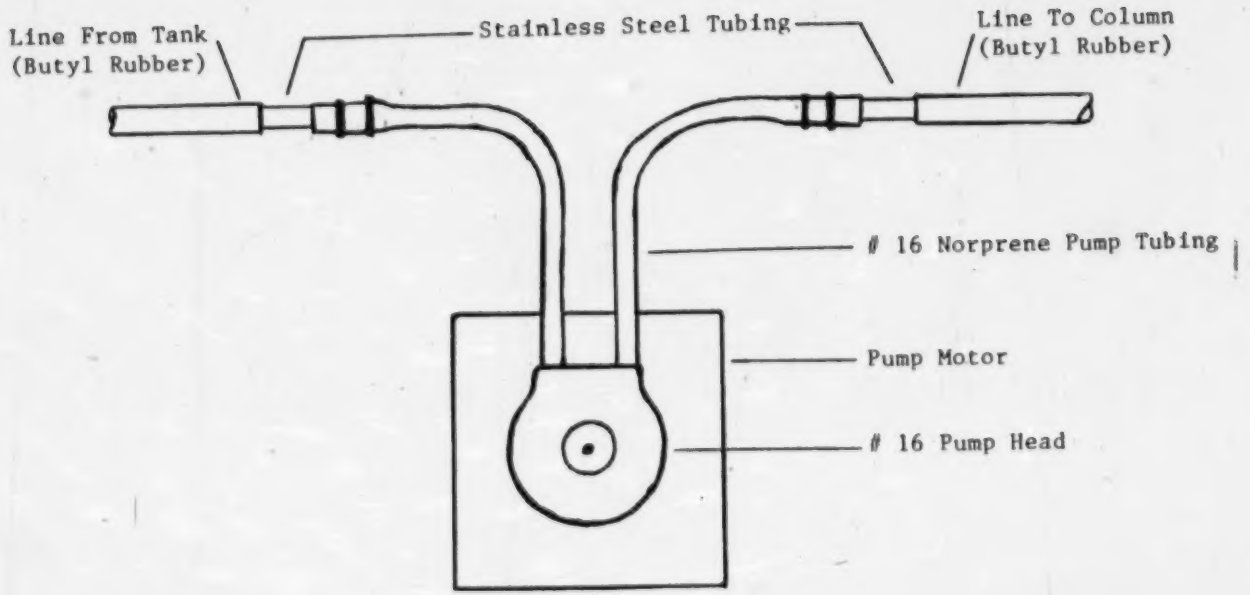


FIGURE I.3: Recirculation Pump and Connections

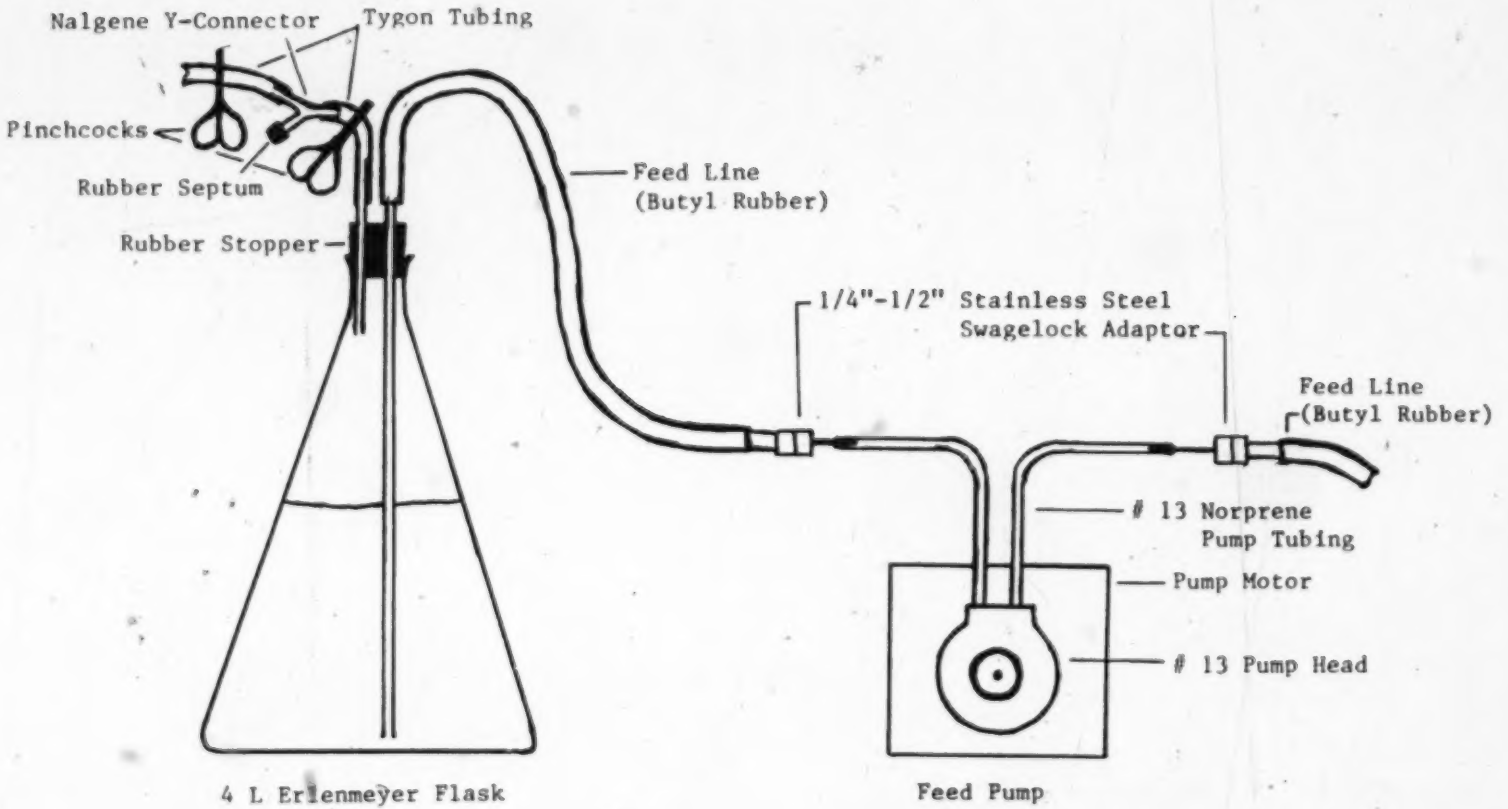


FIGURE I.4: **Feed System**

Figure I.4. The third end of the Y–type connector was sealed by a rubber septum. The Tygon tube sections were clamped with Day pinchcocks. This arrangement was used to pressurize the flask during feed preparation and to add make–up gas to the flask during normal operation. Plastic cable ties were used to secure the rubber stopper in the top of the flask.

I.4 EFFLUENT COLLECTION SYSTEM

Effluent flowrate was regulated by the overflow of liquid from the tank. The effluent overflow and collection system was designed so that a constant liquid level was maintained in the tank at the feed flowrates used. It was also desired that the escape of product gas with the effluent and the risk of exposure of the reactor contents to the atmosphere be minimized. The components of the system are shown in Figure I.5.

Effluent was collected in a 16 inch long cylinder (2.5 inch I.D. x 1/8 inch wall) of Lucite plexiglas fixed to a 4 inch x 4 inch x 3/16 inch Lucite base. A rubber stopper through which two pieces of glass tubing were inserted, sealed the top of the effluent collector. A short section of butyl rubber was connected to the effluent overflow on the tank. A longer piece of Tygon plastic tubing connected the free end of the butyl rubber tube and to one of the glass tubes at the top of the collector. The Tygon tubing was arranged in a large U so that a sufficient liquid level was present in each leg of the U during operation to prevent the escape of product gas. The exit line from the the collector was connected to an empty 500–mL Erlenmeyer flask and then to a 1–L Erlenmeyer flask containing 500 mL of tap water by Tygon tubing as shown in Figure I.5. This design enabled the escape of the gas displaced from the collector by the incoming effluent while protecting the reactor from backflows of air that might occur during a system upset. The effluent container had a liquid capacity of 1300 mL.

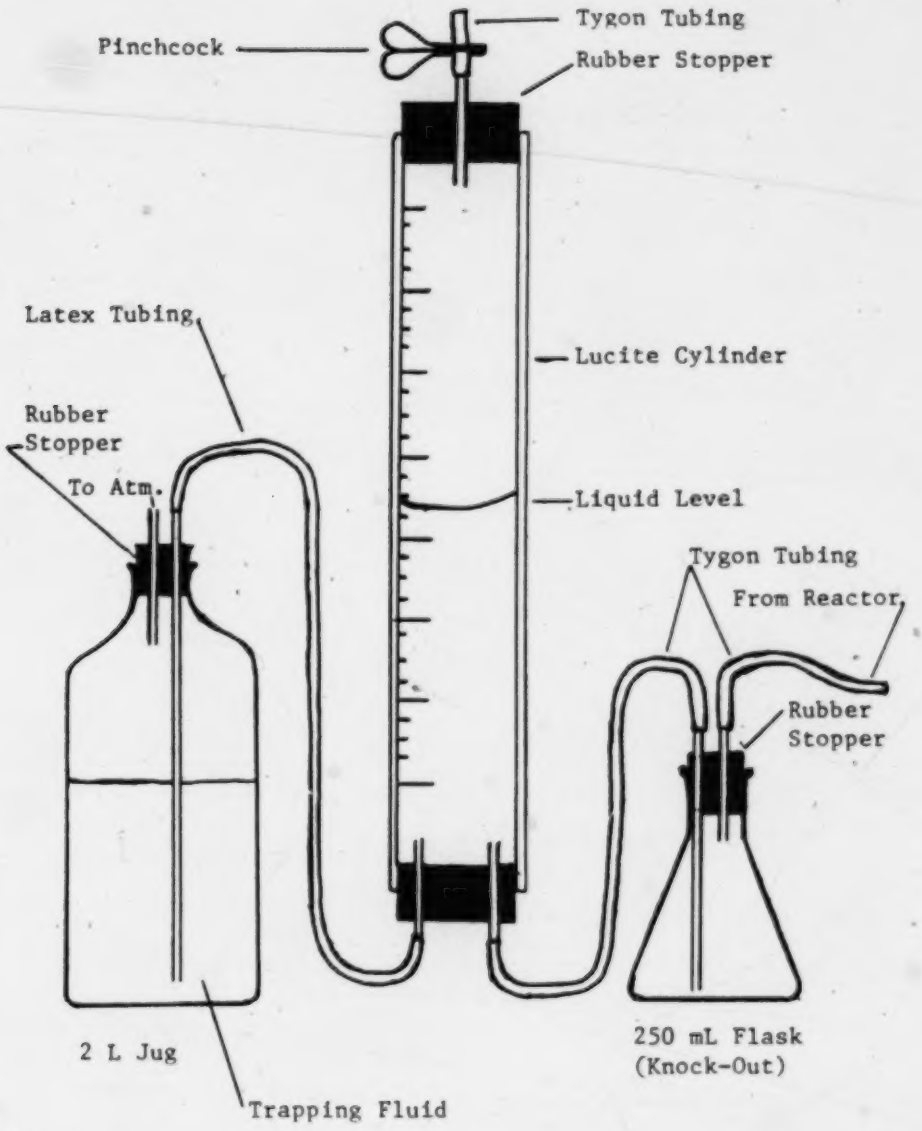


FIGURE I.5: Gas Collection System

I.5 GAS COLLECTION SYSTEM

The carbon dioxide and methane gas produced in the anaerobic phenol degradation reaction was separated from the liquid medium in the reactor tank. The gas escaped through a length of Tygon tubing connected to a glass tube protruding from the top of the tank and was collected and measured in the gas collection system shown in Figure I.6. This system consisted of a knock–out flask, a graduated collection cylinder and a trapping fluid reservoir.

A 250–mL Erlenmeyer flask served as the knock–out flask. Small quantities of liquid escaping from the reactor with the gas were collected in this flask. The flask also offered some protection against the possibility of backflow of trapping fluid into the reactor. The flask was sealed with a rubber stopper through which two lengths of glass tubing were inserted. The Tygon tubing from the tank connected to the shorter inlet tubing. The lower end of the exit tubing was at bottom of the flask and the other end was connected to the gas collection cylinder by a length of Tygon tubing. Normally, the knock–out flask contained very little liquid.

The collection cylinder was a 18 inch length of 2.2 inch x 1/8 inch wall Lucite plexiglas tube which was sealed at both ends by rubber stoppers. Two short lengths of glass tubing were inserted in the bottom stopper; one for the inlet of gas from the knock–out flask, the other to allow the exit of trapping fluid. A single short length of glass tubing was inserted through the stopper at the top end. A short length of Tygon tubing was connected to the upper end of this glass tube and clamped with a Day pinchcock. The collection cylinder was graduated to the nearest 10 mL of volume by adding known volumes of water from a volumetric pipette and marking the level on the Lucite in indelible ink. The volumetric capacity of the cylinder was 1200 mL. The cylinder was held in the vertical position by a support stand.

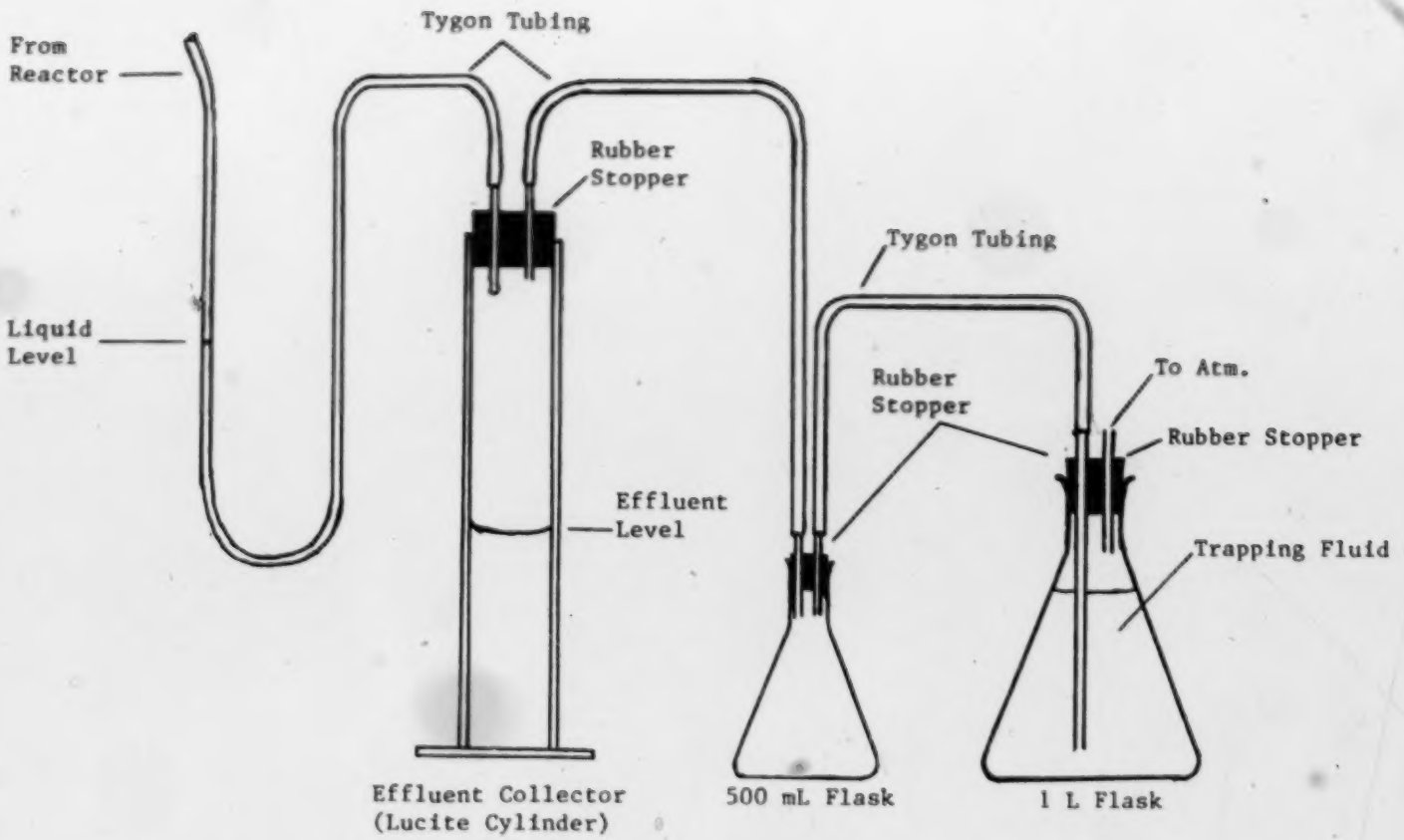


FIGURE I.6: **Effluent Collection System**

Gas entering the collection system from the reactor bubbled up through the trapping fluid in the cylinder and was collected in the headspace above the liquid. Displaced trapping fluid exited the bottom of the cylinder and flowed into the trapping fluid reservoir through a length of flexible latex rubber tubing (5/16 inch O.D.x 1/16 inch wall) The trapping fluid reservoir was a 2–L jug with a rubber stopper inserted in the neck. A long section of glass tubing was inserted through the stopper. The lower end of this glass tube was very near the bottom of the jug. The upper end was attached to the latex tubing from the collection cylinder. A shorter tube was inserted through the same stopper and exposed the headspace of the reservoir to the atmosphere. The trapping fluid was a solution of 200 mg/L sodium chloride and 5 g/L of citric acid in tap water. The high salt content and low pH (*e.g.* 1.7) reduced the solubility of carbon dioxide and methane gases in the trapping fluid. A total of 1.5 L of trapping fluid was contained in the system.

To make a measure of the quantity of gas produced over a period of time, the following procedure was used:

1. The Tygon tubing between the knock–out flask and the bottom of the gas was clamped by a Day pinchcock.
2. The jug was raised so that the liquid level in the column and the liquid level in the jug coincided and were at equilibrium. The pressure of the gas in the headspace of the column was atmospheric at this point.
3. The volume indicated on the cylinder and the atmospheric pressure were recorded.
4. The pinchcock referred to in step one was removed.

The absolute quantity of gas collected was calculated after correcting for moisture content and variations in atmospheric pressure. After a time, the volumetric capacity of the cylinder was exhausted and the system was relevelled. The

procedure was:

1. Steps 1 – 3 of the measuring process were repeated.
2. The pinchcock on the Tygon tube at the top of the cylinder was removed.
3. The jug was raised so that the liquid level in the cylinder rose to the top and equilibrated with the level in the jug. Collected gas was forced out through the opening at the top. The new volume was recorded.
4. The pinchcock at the top of the cylinder was replaced and the jug was lowered.
5. The pinchcock on the entrance line to the bottom of the cylinder was removed.

I.5 SAMPLING SYSTEMS

I.5.1 Gas Samples

A 1/4 inch Nalgene plastic Y–type connector was inserted into the Tygon tubing connecting the tank and the gas collection system at a point near the tank. The third end of the Y–type connector was fitted with a rubber septum. Headspace gas samples were withdrawn by inserting the needle of a 0.5–mL single–dose insulin syringe through the septum and drawing back the plunger to withdraw 0.2 mL of gas. The needle of the syringe was withdrawn from the septum and quickly inserted into a butyl–rubber stopper. The syringe was then transported to the GC and the sample was analyzed for methane Section 4.6.

I.5.2 Liquid Samples

The liquid sampling system is shown in Figure I.7. A 10–cm length of Tygon tubing was connected to the glass sample tube at the top of the tank. The other end of this piece of tubing was connected to a 1/4 inch Nalgene plastic Y–type

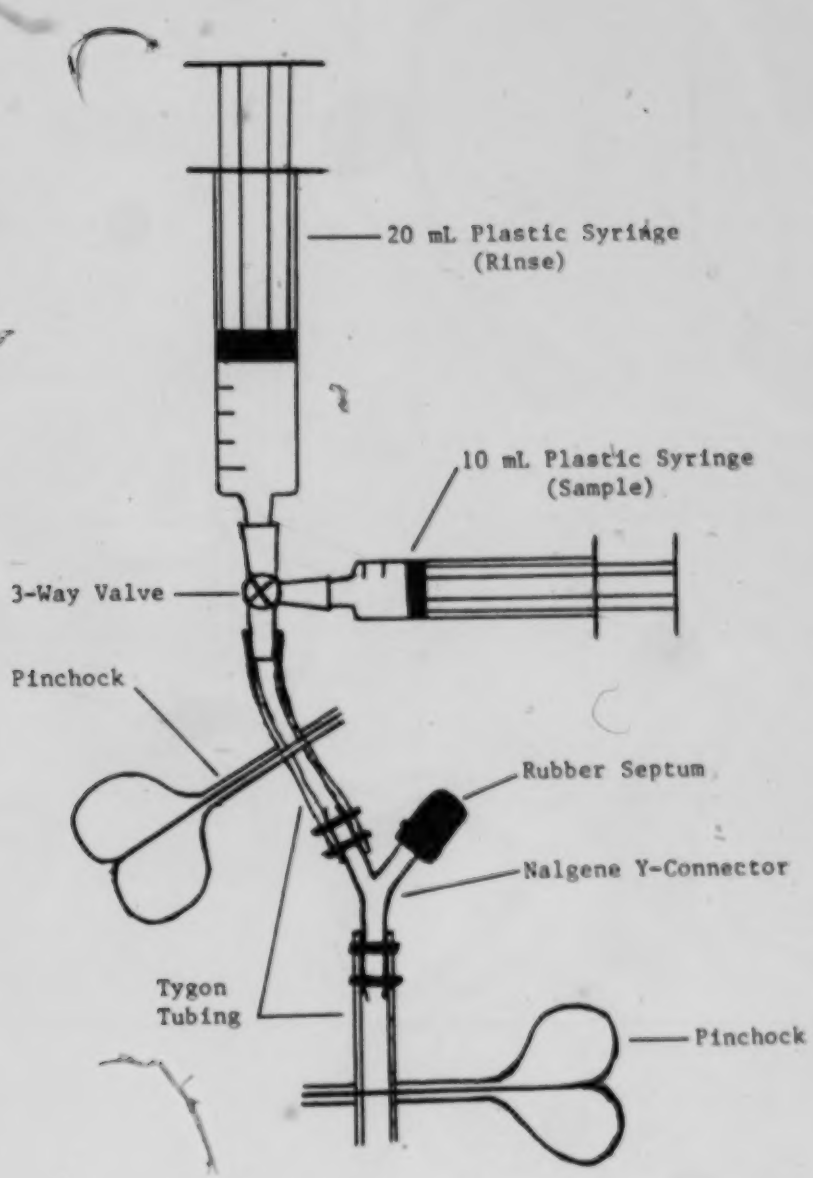


FIGURE I.7: Liquid Sampling System

connector. Another 10–cm length of Tygon tubing was connected to one end of the Y–type connector. A rubber septum was attached to the other end of the Y–type connector. During normal reactor operation both sections of Tygon tubing were clamped with Day pinchcocks.

An apparatus consisting of a 10–mL plastic syringe, a 20–mL plastic syringe and a 3–way plastic valve with two female ends and one male end, was used, in conjunction with the arrangement described in the previous paragraph, to obtain a liquid sample. The sampling procedure is described as follows:

1. Each syringe (with plunger depressed) was inserted snugly into one of the fittings of the 3–way valve. The male end of the valve was then fitted into into the open end of the Tygon tubing.
2. The effluent and gas lines from reactor were clamped with Day pinchcocks and the two pinchcocks on the sample line were removed.
3. With the 3–way valve was turned so that the 10–mL sample syringe was isolated, the sample line was flushed by drawing 15 mL of medium into the 20–mL syringe.
4. The 20–mL flushing syringe was then isolated and a sample was drawn into the 10–mL syringe.
5. The sample syringe was again isolated and the 15 mL contents of the 20–mL syringe were returned to the reactor.
6. Steps 2 and 1 were reversed, in that order, and the sample was ejected from the 10–mL syringe into a 5–mL test–tube. The test tube was sealed with Parafilm.

The sample system was also used for addition of reduced liquids (*i.e.* concentrated phenol solution, sodium sulphide solution). The 3–way valve and the sample syringe were not required. The procedure was:

1. The 20–mL flushing syringe was inserted into the end of the Tygon sample tube tubing.
2. Same as step 2 of sampling procedure.
3. 15 mL of tank contents were drawn into the 20–mL syringe and the Tygon tubing was clamped above the Y–type connector.
4. The reduced liquid, transferred from a sealed serum bottle via a syringe and 22 1/2 gauge needle, was injected through the rubber septum.
5. The Tygon tube was unclamped and the contents of the 20–mL syringe were returned to the reactor, flushing the contents of the sample line into the reactor.
6. Steps 2 and 1 were reversed, in that order.

APPENDIX II DATA AND CALCULATIONS FOR BIOREACTOR STUDY

APPENDIX II.A PSEUDO STEADY–STATE CFRB DATA

TABLE II.A.1 CFRB DATA: RUN 1

FEED PHENOL CONCENTRATION: 639 mg/L

Day	mL effluent collected	Feed Flowrate (mL/d)	mmoles dry gas collected	Conc. (mg/L) Phenol	Acetate
0	0	—	0.0	13	185
1	382	344	0.4	100	203
2	690	341	4.2	90	—
3	1037	376	7.9	81	256
4	1345	282	12.3	80	—
5	1657	322	17.6	75	267
6	1922	326	19.6	69	—
7	2305	332	25.8	74	286
8	2745	346	30.9	60	—
9	3037	336	33.9	51	330

TOTAL DURATION OF RUN:	220	h
TOTAL EFFLUENT COLLECTED:	3037	mL
TOTAL DRY GAS COLLECTED:	33.6	mole
AVERAGE FEED FLOWRATE:	332	mL/d
AVERAGE DRY GAS PRODUCTION:	4.3	mmole/d
FINAL PHENOL CONC.:	51	mg/L
FINAL HAc:	330	mg/L
FINAL HEADSPACE CH_4 CONC.:	75 %	

TABLE II.A.2 CFRB DATA: RUN 2

FEED PHENOL CONCENTRATION: 803 mg/L

Day	mL effluent collected	Feed Flowrate (mL/d)	mmoles dry gas collected	Conc. (mg/L) Phenol	Acetate
0	0	—	0.0	0	0
1	312	273	1.8	176	33
2	585	364	4.8	219	—
3	1037	366	8.3	229	188
4	1275	342	13.2	230	—
5	1790	360	19.2	231	281
6	—	—	—	228	—
7	2484	342	22.2	227	303
8	2694	335	25.7	219	—
9	3019	337	30.4	208	292

TOTAL DURATION OF RUN:	211	h
TOTAL EFFLUENT COLLECTED:	3019	mL
TOTAL DRY GAS COLLECTED:	30.4	mole
AVERAGE FEED FLOWRATE:	343	mL/d
AVERAGE DRY GAS PRODUCTION:	3.5	mmole/d
FINAL PHENOL CONC.:	208	mg/L
FINAL HAc CONC.:	292	mg/L
FINAL HEADSPACE CH_4 CONC.:	75	%

TABLE II.A.3 VSS CONCENTRATION IN EFFLUENT COLLECTED

RUN	DAY EFFLUENT COLLECTED	VSS (mg/L)
1	2	34
1	6	20
1	9	31
2	3	12
2	5	13
2	9	17

AVERAGE FOR RUN 1: 28 mg/L

AVERAGE FOR RUN 2: 14 mg/L

APPENDIX II.B CFRB DATA: BATCH PHASE

TABLE II.B.1 BATCH DEGRADATION DATA: CFRB RUN 1

Tank Fed 3.5 mL of 29 400 mg/L Conc. Phenol Stock Soln. at Time = –0.35 h

Time (h)	Phenol (mg/L)
0	176.1
1.55	153.6
3.33	136.9
5.07	119.8
6.60	110.0

DEGRADATION RATE (Determined from linear least squares fit):

10.1 mg/L/h (correlation of fit: 0.992)

MEASURED TANK VSS CONCENTRATION (Mean of triplicate determination):

1050 mg VSS/L (s = 89 mg VSS/L)

AVERAGE LIQUID HOLD–UP DURING EXPERIMENT: 465 ± 3 mL

PHENOL DEGRADATION RATE IN TANK:

$V_f = (0.465\ L)(10.1\ mg/L/h)(24\ h/d) = 112.7\ mg/d$

TOTAL BIOMASS IN TANK (VSS + WALL–GROWTH):

$m_f = (0.465\ L)(1050\ mg\ VSS/L) + 90\ mg = 578\ mg$

TABLE II.B.2 BATCH DEGRADATION DATA: CFRB RUN 2

Tank Fed 2 mL of 29 400 mg/L Conc. Phenol Stock Soln. at Time = –1.33 h

Time (h)	Phenol (mg/L)
0	270.0
3.58	224.4
7.48	175.8
10.53	141.4
13.75	105.8

DEGRADATION RATE (Determined from linear least squares fit):

11.96 mg/L/h (correlation of fit: –0.9995)

MEASURED TANK VSS CONCENTRATION (Mean of triplicate determination):

1370 mg VSS/L (s = 110 mg VSS/L)

AVERAGE LIQUID HOLD–UP DURING EXPERIMENT: 466 ± 3 mL

PHENOL DEGRADATION RATE IN TANK:

V_f = (0.466 L)(11.96 mg/L/h)(24 h/d) = 133.8 mg/d

TOTAL BIOMASS IN TANK (VSS + WALL–GROWTH):

m_f = (0.466 L)(1370 mg VSS/L) + 90 mg = 728 mg

APPENDIX II.C CALCULATIONS FOR CFRB KINETIC ANALYSIS

RUN 1:

ABSOLUTE DEGRADATION KINETICS:

1. **OVERALL REACTOR:**

from eqn. 5.1:

$$V_o = F(C_o - C)$$
$$= (0.332\ L/d)(639\ mg/L - 51\ mg/L)$$
$$= 195.2\ mg\ phenol/d$$

2. **BATCH TANK DEGRADATION:**

$$V_f = 112.7\ mg/L$$

3. **GAC–ATTACHED CULTURE:**

from eqn. 5.7:

$$V_c = V_o - V_f$$
$$= 195.2\ mg/L - 112.7\ mg/L$$
$$= 82.5\ mg/L$$

SPECIFIC DEGRATION RATES:

1. **OVERALL REACTOR:**

from eqn. 5.4:

$$\nabla_o = V_o/m_b$$
$$= (195.2\ mg\ phenol/L)/(1097\ mg\ VS)$$
$$= 0.178\ mg\ phenol/mg\ VS/d$$

2. **FLOCCULANT BIOMASS (including wall growth):**

from eqn. 5.5:

$$\nabla_f = V_f/m_f$$
$$= (112.7\ mg\ phenol/L)/(578\ mg\ VS)$$
$$= 0.194\ mg\ phenol/mg\ VS/d$$

3. GAC–ATTACHED CULTURE:

mass of GAC in reactor, $m_c = 5.1$ g

concentration of biomass on GAC (from TGA), $x_{b/c} = 101$ mg VS/g GAC

from eqn. 5.6:

$$\nabla_c = V_c/(m_c \cdot x_{b/c})$$

$$= (82.5 \text{ mg phenol/L})/(5.1 \text{ g GAC} \cdot 101 \text{ mg VS/g GAC})$$

$$= 0.160 \text{ mg phenol/mg VS/d}$$

RUN 2:

ABSOLUTE DEGRADATION KINETICS

1. OVERALL REACTOR:

from eqn. 5.1:

$$V_o = F(C_o - C)$$

$$= (0.343 \text{ L/d})(803 \text{ mg/L} - 208 \text{ mg/L})$$

$$= 204.1 \text{ mg phenol/d}$$

2. BATCH TANK DEGRADATION:

$$V_f = 133.8 \text{ mg/L}$$

3. GAC–ATTACHED CULTURE:

from eqn. 5.7:

$$V_c = V_o - V_f$$

$$= 204.1 \text{ mg/L} - 133.8 \text{ mg/L}$$

$$= 70.3 \text{ mg/L}$$

SPECIFIC DEGRADATION RATES:

1. **OVERALL REACTOR:**

from eqn. 5.3: $\nabla_o = V_o/m_b$

$= (204.1 \text{ mg phenol/L})/(1246 \text{ mg VS})$

$= 0.164 \text{ mg phenol/mg VS/d}$

2. **FLOCCULANT BIOMASS (including wall growth):**

from eqn. 5.5: $\nabla_f = V_f/m_f$

$= (133.8 \text{ mg phenol/L})/(728 \text{ mg})$

$= 0.184 \text{ mg phenol/mg VS/d}$

3. **GAC–ATTACHED CULTURE:**

mass of GAC in reactor, $m_c = 5.1 \text{ g}$

concentration of biomass on GAC (from TGA), $x_{b/c} = 101 \text{ mg VS/g GAC}$

from eqn. 5.4:

$\nabla_c = V_c/(m_c \cdot x_{b/c})$

$= (70.3 \text{ mg phenol/L})/(5.1 \text{ g GAC} \cdot 101 \text{ mg VS/g GAC})$

$= 0.137 \text{ mg phenol/mg VS/d}$

APPENDIX II.D DATA AND CALCULATIONS FOR 2–L DIGESTER

TABLE II.D.1 DATA FROM BATCH DEGRADATION

DIGESTER FED AT TIME = –5 min

Time(h)	Phenol (mg/L)	mmoles dry gas collected
0.0	239.0*	0.0
2.13	228.9*	0.33
4.02	217.3*	1.17
6.17	200.2	2.95
8.38	178.3	4.85
10.95	155.1	7.40
14.50	113.4	10.89
19.27	59.7	15.39
21.50	36.2	17.62
23.45	13.3	18.56
24.47	3.3	19.51

BATCH DEGRADATION RATE (by linear least squares fit to data):

$V_s/V_l = 10.96$ mg /L/h (correlation of fit = –0.9996)

* data ommitted from least squares fit

MEASURED VSS CONCENTRATION (mean of triplicate determinations):

$C_s = 1700$ mg VSS/L (std. deviation = 33 mg/L)

SPECIFIC DEGRADATION RATE OF SUSPENDED CELLS, ∇_s:

from eqn. 5.6: $\nabla_s = (V_s/V_l)/C_s$

$\nabla_s = \left[(10.96 \text{ mg phenol/L/h})/(1700 \text{ mg VSS/L})\right](24 \text{ h/d})$

$= 0.15$ mg phenol/mg VSS/d

APPENDIX II.E ANALYSIS OF CSTR DYNAMICS

This analysis shows that after five residence times following a step change in feed concentration in a CSTR the transient is essentially complete. The mass balance on an ideal CSTR with zero order reaction rate is

$$V\frac{dC}{dt} = F(C_o - C) \qquad (II.1)$$

where C is concentration of the reactant in the reactor, C_o is feed concentration, V is reactor volume, F is flowrate and R is the zero order reaction rate expression. Consider two steady–states, 1 and 2, which exist before and after a step–change in feed concentration. The mass balance equations representing these steady–states are

$$0 = F(C_{o,1} - C_{ss,1}) - R \qquad (II.2)$$

$$0 = F(C_{o,2} - C_{ss,2}) - R \qquad (II.3)$$

where C_{ss} is the steady–state reactor concentration. The difference of II.2 and II.3 gives

$$(C_{ss,2} - C_{ss,1}) = (C_{o,2} - C_{o,1}) \qquad (II.4)$$

Equations II.1 and II.2 can be solved by the method of Laplace transforms for a

step–change in feed concentration of magnitude $(C_{o,2} - C_{o,1})$. The solution is

$$\frac{C(t) - C_{ss,1}}{C_{o,2} - C_{o,1}} = 1 - e^{-t/\tau} \qquad \text{(II.5)}$$

where C(t) is the reactor concentration at a time following the step change and τ is the average residence time of the reactor. For a CSTR the average residence time is equal to the hydraulic residence time which is given by the ratio V/F. Substituting II.4 into II.5 gives

$$\frac{C(t) - C_{ss,1}}{C_{ss,2} - C_{ss,1}} = 1 - e^{-t/\tau} \qquad \text{(II.6)}$$

The term on the left–hand side represents the fraction of the final change in the reactor concentration that has taken place at time t following the step–change in feed concentration. At time equals infinity, this term is equal to 1 and the the transient is complete. After 5 hydraulic residence times, *e.g.* $t = 5\tau$, the fraction, as predicted by eqn. II.6, is

$$\frac{C(t) - C_{ss,1}}{C_{ss,2} - C_{ss,1}} = 1 - e^{-5} = 0.993$$

In other words, the transient resulting from the disturbance in feed concentration is 99.3% complete.

APPENDIX II.F PSEUDO STEADY–STATE ERROR ANALYSIS

An estimate of the order of the error involved in determining the overall bioreactor activity from pseudo steady–state data is calculated in this section. The sources of error considered are:

1. Increasing biological activity in the reactor with time.
2. Desorption of phenol from the GAC with time.

1. CFRB RUN 1:

TABLE II.F.1 PHENOL CONC. DATA: LAST 3 DAYS OF CFRB RUN 1

DAY	TIME (h)	PHENOL (mg/L)
7	0	74
8	30.5	60
9	51.4	51

Estimate of error due to increasing phenol degrading activity:

– Rate of decrease in phenol concentration in last 3 days of the run was estimated by a linear least squares fit of the data of Table II.F.1:

10.8 mg/L/d, (correlation of fit: –0.99985)

– Absolute rate of decrease: (10.8 mg/L/d)(0.608 L) = 6.7 mg/d

– Overall reactor degradation rate (Table 5.2): 195 mg/d

– estimate of percent error: [(6.7 mg/d)/(195 mg/d)] x 100% = 3.4%

Estimate of error due to desorption:

– A linearized rate of desorption was estimated using phenol concentration data from Table II.F.1 and the equilibrium relationship for the adsorption of phenol onto biomass free GAC (Kindzierski, 1989):

Phenol adsorption isotherm (from equation 5.10):

$$q = 44.4\ C_e^{0.17}$$

Linearized rate of adsorption:

$$= \frac{(5.1\ g\ GAC)(44.4\ mg\ phenol/g\ GAC)[\ 74^{0.17} - 51^{0.17}](24\ h/d)}{(54.4\ h)}$$

$$= 13.5\ mg/d$$

% error in overall degradation rate:

$$[(13.5\ mg/d)/(195\ mg/d)] \times 100\% = 6.9\%$$

Estimate of Total Error: $3.4\% + 6.9\% = 10.3\%$

Error in determining the overall bioreactor activity rate was, therefore, on the order of 10%.

2. CFRB RUN 2:

TABLE II.F.2 PHENOL CONC. DATA: LAST 3 DAYS OF CFRB RUN 2

DAY	TIME (h)	PHENOL (mg/L)
7	0	227
8	15.1	219
9	38.1	208

Estimate of error due to increasing phenol degrading activity:

– Rate of decrease in phenol concentration in last 3 days of the run was estimated by a linear least squares fit of the data of Table II.F.2:

11.5 mg/L/d, (correlation of fit: –0.9996)

– Absolute rate of decrease: (11.5 mg/L/d)(0.608 L) = 7.0 mg/d

– Overall reactor degradation rate (Table 5.2): 204 mg/d

– estimate of percent error: [(7.0 mg/d)/(204 mg/d)] x 100% = 3.4%

Estimate of error due to desorption:

– A linearized rate of desorption was estimated using phenol concentration data from Table II.F.2 and the equilibrium relationship for the adsorption of phenol onto biomass free GAC (Kindzierski, 1989).

Linearized rate of adsorption:

$$= \frac{(5.1\ \text{g GAC})(44.4\ \text{mg phenol/g GAC})[\ 227^{0.17} - 208^{0.17}](24\ \text{h/d})}{(38.1\ \text{h})}$$

$= 5.3$ mg/d

% error in overall degradation rate:

$$[(5.3\ \text{mg/d})/(204\ \text{mg/d})] \times 100\% = 2.6\%$$

Estimate of Total Error: $3.4\% + 2.6\% = 6.0\%$

Error in determining the overall bioreactor activity rate was, therefore, on the order of 6%.

APPENDIX II.G PHENOL BALANCE CALCULATIONS

II.G.1 CALCULATION OF PHENOL EQUIVALENCE CONVERSIONS

1.Phenol to CH_4 Gas

Phenol is assumed to be converted to CH_4 and CO_2 according to the stoichiometry of equation 3.1:

$$C_6H_6O + 4\ H_2O \longrightarrow 7/2\ CH_4 + 5/2\ CO_2 \qquad (3.1)$$

One mole of phenol produces 7/2 a mole of CH_4 gas. Using the molecular weights of phenol and CH_4:

1 gmole phenol ⟶ 7/2 gmole CH_4

(1 gmole phenol)(94 g/gmole) ⟶ (7/2 gmole CH_4)(16 g/gmole)

94 g phenol ⟶ 56 CH_4

1 g phenol ⟶ 0.596 g CH_4

1 mg phenol ⟶ 0.596 mg CH_4

2. Phenol to Acetic Acid

The conversion of phenol to acetic acid is assumed to proceed according to the stoichiometry of equation 3.3:

$$C_6H_6O + 5\ H_20 \longrightarrow 3\ CH_3COOH + 2\ H_2 \qquad (3.3)$$

1 gmole phenol ⟶ 3 gmoles acetic acid

(1 gmole phenol)(94 g/gmole) ⟶ (3 gmoles acetic acid)(60 g/gmole)

94 g phenol ⟶ 180 g acetic acid

1 g phenol ⟶ 1.91 g acetic acid

1 mg phenol ⟶ 1.91 mg acetic acid

3. Phenol to Biomass conversion

Elemental analysis of anaerobic phenol degrading biomass from the 2–L digester was done by the Microanalytical Laboratory in the Department of Chemistry at the University of Alberta, Edmonton, Alberta. Biomass–containing medium from the digester was centrifuged at 1000 rpm for 5 min. The supernatant liquid was decanted from the centrifuge tube and replaced with dd H_2O. The biomass was re–suspended and the centrifuging and decanting step was repeated twice more. The final biomass pellet was freeze dried overnight ($T = -60^{\circ}C$, $P < 10$ microns Hg) and then delivered for analysis. The analysis is shown in Table II.G.1 along with the elemental composition of a typical microbial cell (Stanier *et al.*, 1976) for comparison.

TABLE II.G.1 ELEMEMTAL COMPOSITION OF BIOMASS

ELEMENT	ANAEROBIC BIOMASS	TYPICAL CELL
% C	44.8	50
% H	6.4	8
% N	8.5	14
% S	2.1	1
% O	38.2	20

The approximate molecular formula derived from the above analysis and the atomic weights of the elements involved is:

$$C_1 H_{1.71} N_{.16} S_{.02} O_{.64}$$

The formula molecular weight per mole of carbon is 26.9 g/gmole. The corresponding results for typical microbial cells (based on the information of

Table II.G.1) are:

$$C_1 H_{1.92} N_{.21} S_{.01} O_{.30}$$

and 22 g/gmole. Compared to literature values of bacterial composition the results of the elemental analysis of the anaerobic biomass are quite reasonable and are are acceptable for use in this mass balance calculation. If phenol is the sole carbon source from which new cell material is synthesized, then each mole of phenol incorporated will produce 6 formula equivalents of biomass, or

(1 gmole phenol)(94 g/gmole) ⟶ (6 equivalents biomass)(26.9 g/gmole)

94 g phenol ⟶ 161.4 g biomass

1 g phenol ⟶ 1.71 g biomass

1 mg phenol ⟶ 1.71 mg biomass

II.G.2 MASS BALANCE ON PHENOL

Mass balances were performed in terms of phenol equivalents using the data from the continuous phases of the CFRB runs (Tables II.A.1, II.A.2, II.A.3, II.H.1) and the estimations of the flocculant biomass quantity determined during the batch phases. The mass balance equation for a run is:

PHENOL IN = PHENOL OUT + CH_4 PRODUCED + ACETIC ACID PRODUCED + BIOMASS PRODUCED (II.G.1)

CH_4, acetic acid and biomass production were converted into phenol equivalents using the previously derived relationships.

RUN O/1

– The mass balance for this run was done using data from chemostat Run 1 (Table II.A.1, Table II.A.3) and add additional data from trial chemostat Run 0 (Table II.H.1, Table II.H.2)

1. **Phenol in**

– estimated from volume of feed delivered and feed concentration

1st feed flask (Run no. 0): $C_f = 442$ mg/L, $V_f = 3.060$ L

2nd feed flask (Run no. 1): $C_f = 639$ mg/L, $V_f = 3.037$ L

Total Phenol in: (442 mg/L)(3.060 L) + (639 mg/L)(3.037 L) = 3293 mg

2. **Phenol out**

–Phenol in effluent was estimated by multiplying the daily recorded flowrates by the phenol concentration of the daily samples (Table II.A.1, Table II.H.1). Phenol hold–up at the end of the run was estimated from the liquid hold–up and the final concentration.

Total Phenol out: (285 mg) + (0.608)(51 mg) = 316 mg

3. **Phenol to CH_4**

– estimated from total mmoles of dry gas collected during run and CH_4 content:

Total dry gas produced: 46.3 mmoles

CH_4 content: 75%

CH_4 produced: $\dfrac{(.75)(46.3 \text{ mmoles})(16 \text{ mg/mmoles})}{(0.596 \text{ g } CH_4/\text{g phenol})}$

= 932 mg phenol equivalent.

4. **Acetic Acid Produced**

– estimated in same manner as phenol out.

$$\text{Acetic Acid out: } \frac{(1208 \text{ mg}) + (0.608 \text{ L})(330 \text{ mg/L})}{(1.91 \text{ mg acetic acid/mg phenol})}$$

= 737 mg phenol equivalent

5. **Biomass Produced**

– estimated from measurements of flocculant and suspended biomass at the beginning and the end of the run measurement and of biomass concentration in the effluent. Corrections were made for the amount of biomass which accumulated in the effluent lines during the run (this biomass was collected and the quantity determined) and for the biomass lost in sampling the tank for a VSS determination.

Initial holdup of flocculant biomass (start of Run No. 0): 305 mg

Initial holdup of suspended biomass (start of Run No. 0): 5 mg

Final holdup of flocculant biomass (end of Run No. 1): 475 mg

Final holdup of suspended biomass (end of Run No. 1): 17mg

Biomass collected in effluent : 153 mg

Biomass accumulated in effluent lines: 44 mg

Biomass in VSS sample: 15 mg

Total biomass produced: (475 mg + 17 mg + 153 mg + 44 mg + 15 mg)

$$-(305 \text{ mg} + 5 \text{ mg}) = 394 \text{ mg}$$

Total phenol equivalent: (394 mg biomass)/(1.71 mg biomass/mg phenol)

= 230 mg

6. **Closure**

– substituting calculated values into equation II.G.1:

Phenol in = 3293 mg

Phenol out = 316 mg + 932 mg + 737 mg + 230 mg = 2215 mg

Phenol unaccounted for: 3293 mg – 2215 mg = 1078 mg

% deviation from closure: (1078 mg/3293 mg) x 100% = 33%

7. **Biomass yield**

– mass of biomass produced per mass of phenol degraded:

$$Y_{b/s} = \frac{\text{biomass produced}}{\text{phenol out} - \text{phenol in}} = \frac{394 \text{ mg}}{3293 \text{ mg} - 316 \text{ mg}}$$

$$= 0.13 \frac{\text{mg VSS}}{\text{mg phenol}}$$

RUN 2

1. **Phenol in**

– estimated from volume of feed delivered and feed concentration

1st (and only) feed flask: $C_f = 803$ mg/L, $V_f = 3.019$ L

Total Phenol in: (803 mg/L)(3.019 L) = 2424 mg

2. **Phenol out**

–Phenol in effluent was estimated by multiplying the daily recorded flowrates by the phenol concentration of the daily samples (Table II.A.2). Phenol hold–up at the end of the run was estimated from the liquid hold–up and the final concentration.

Total Phenol out: (692 mg) + (0.608 L)(208 mg/L) = 818 mg

3. **Phenol to CH_4**

– estimated from total mmoles of dry gas collected during run and CH_4 content:

Total dry gas produced: 30.4 mmoles

CH_4 content: 75%

CH_4 produced: $\frac{(.75)(30.4 \text{ mmoles})(16 \text{ mg/mmoles})}{(0.596 \text{ g } CH_4/\text{g phenol})}$

= 612 mg phenol equivalent.

4. **Acetic Acid Produced**

– estimated in same manner as phenol out.

Acetic Acid out: $\frac{(652 \text{ mg}) + (0.608 \text{ L})(292 \text{ mg/L})}{(1.91 \text{ mg acetic acid/mg phenol})}$

= 434 mg phenol equivalent

5. **Biomass Produced**

–estimated from measurements of flocculant and suspended biomass at the beginning and the end of the run and biomass concentration in the effluent. Corrections were made for the amount of biomass which accumulated in the effluent lines during the run (this biomass was collected and the quantity determined) and for the biomass lost in sampling the tank for a VSS determination.

Initial holdup of flocculant biomass (start of Run No. 2): 475 mg

Initial holdup of suspended biomass (start of Run No. 2): 17 mg

Final holdup of flocculant biomass (end of Run No. 2): 625 mg

Final holdup of suspended biomass (end of Run No. 2): 9 mg

Biomass collected in effluent : 43 mg

Biomass accumulated in effluent lines: 57 mg

Biomass in VSS sample: 21 mg

Total biomass produced: (625 mg + 9 mg + 43 mg + 57 mg + 21 mg)

$$-(475 \text{ mg} + 17 \text{ mg}) = 263 \text{ mg}$$

Total phenol equivalent: (263 mg biomass)/(1.71 mg biomass/mg phenol)

$$= 154 \text{ mg}$$

6. **Closure**

–substituting calculated values into equation II.G.1:

Phenol in = 2424 mg

Phenol out = 818 mg + 612 mg + 434 mg + 154 mg = 2018 mg

Phenol unaccounted for: 2424 mg – 2018 mg = 406 mg

% deviation from closure: (406 mg/2424 mg) x 100% = 17%

7. **Biomass yield**

– mass of biomass produced per mass of phenol degraded:

$$Y_{b/s} = \frac{\text{biomass produced}}{\text{phenol out} - \text{phenol in}} = \frac{154 \text{ mg}}{2424 \text{ mg} - 818 \text{ mg}}$$

$$= 0.10 \frac{\text{mg VSS}}{\text{mg phenol}}$$

APPENDIX II.H ADDITIONAL DATA FOR PHENOL BALANCE

TABLE II.H.1 DATA FROM CFRB RUN 0

FEED PHENOL CONCENTRATION: 442 mg/L

Day	mL effluent collected	Feed Flowrate (mL/d)	mmoles dry gas collected	Conc. (mg/L) Phenol	Acetate
0	0	—	0.0	7	N.D.
1	109	119	0.3	—	—
2	281	148	—	8	13
3	441	197	1.1	—	—
4	655	193	3.1	6	34
5	1360	663	6.7	24	20
6	1610	270	8.3	18	—
7	1941	315	10.5	35	111
8	2331	360	13.9	12	—
9	2604	338	16.5	31	167

TABLE II.H.2 VSS CONCENTRATION IN EFFLUENT: CFRB RUN 0

DAY	VSS CONCENTRATION (mg/L)
4	17
8	24

APPENDIX III SHOCK LOADING EXPERIMENT: ADDITIONAL FIGURES

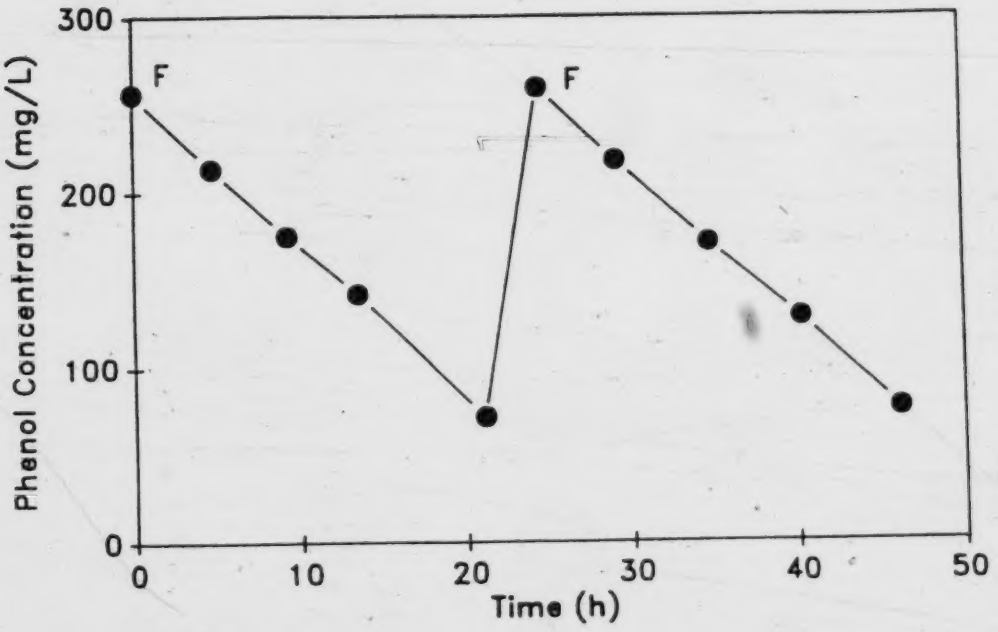


FIGURE III.1: Pre–shock load batch activity in flocculated biomass culture 2 determined in two sequential batch degradations. This is the initial portion (T < 50 h) of the data of Figure 6.2 presented on a magnified scale in order to illustrate the 0 – order kinetic behaviour.

F = pulse feeding at non–inhibitory phenol level (*i.e* < 300 mg/L)

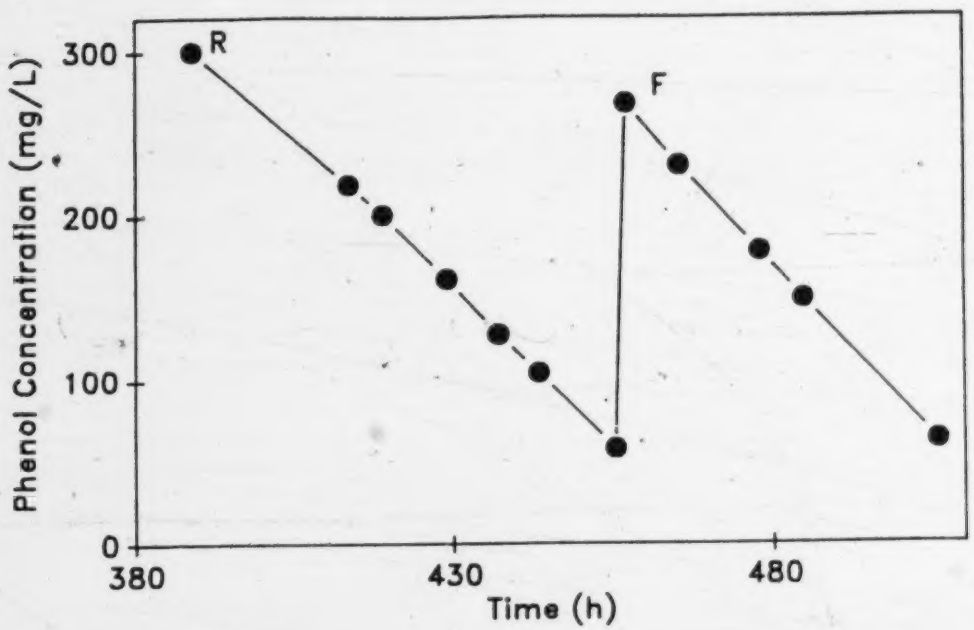


FIGURE III.2: Post–shock load batch activity in flocculated biomass culture 2 determined in two sequential batch degradations. This is the final portion (T > 380 h) of the data of Figure 6.2 presented on a magnified scale in order to illustrate the 0 – order kinetic behaviour.

F = pulse feeding at non–inhibitory phenol level (*i.e* < 300 mg/L)

R = phenol concentration remaining following degradation of phenol added with shock load

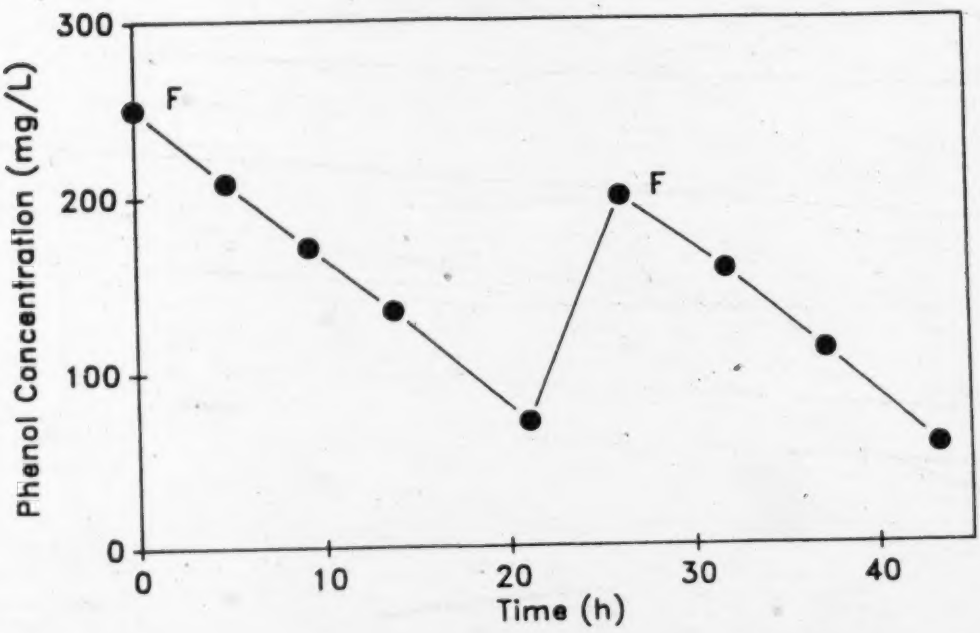


FIGURE III.3: Pre–shock load batch activity in flocculated biomass culture 3 determined in two sequential batch degradations. This is the initial portion (T < 50 h) of the data of Figure 6.3 presented on a magnified scale in order to illustrate the 0 – order kinetic behaviour..

F = pulse feeding at non–inhibitory phenol level (*i.e* < 300 mg/L)

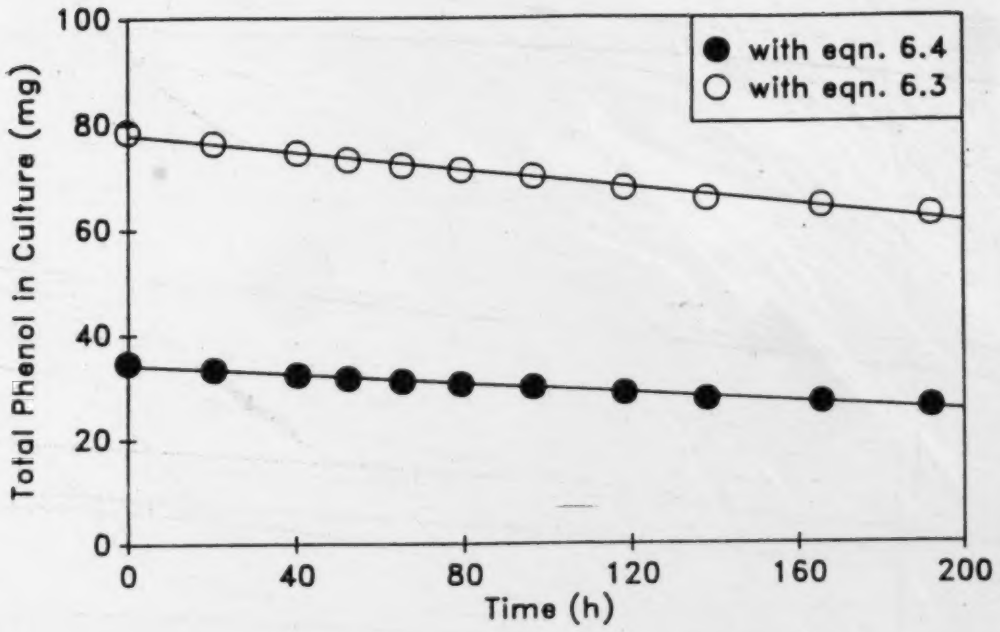


FIGURE III.4: Pre–shock load activity in GAC culture 2. Total phenol in the culture (sum of adsorbed phenol and phenol in supernatant) is on the vertical axis. Top curve is upper bound on total phenol calculated using the experimentally determined phenol adsorption isotherm for clean GAC (equation 6.3). Bottom curve is lower bound on total phenol calculated using the experimentally determined phenol adsorption isotherm for GAC from the bioreactor column (equation 6.4). Linear least–square fits to the data are shown.

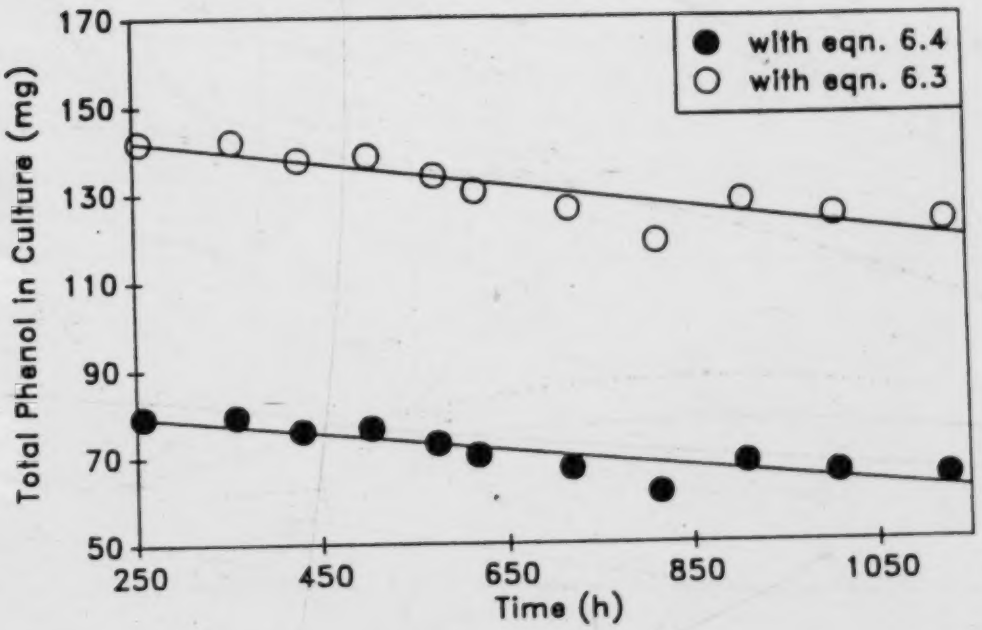


FIGURE III.5: Post–shock load activity in GAC culture 2. Total phenol in the culture (sum of adsorbed phenol and phenol in supernatant) is on the vertical axis. Top curve is upper bound In total phenol calculated using the experimentally determined phenol adsorption isotherm for clean GAC (equation 6.3). Bottom curve is lower bound on total phenol calculated using the experimentally determined phenol adsorption isotherm for GAC from the bioreactor column (equation 6.4). Linear least–square fits to the data are shown.

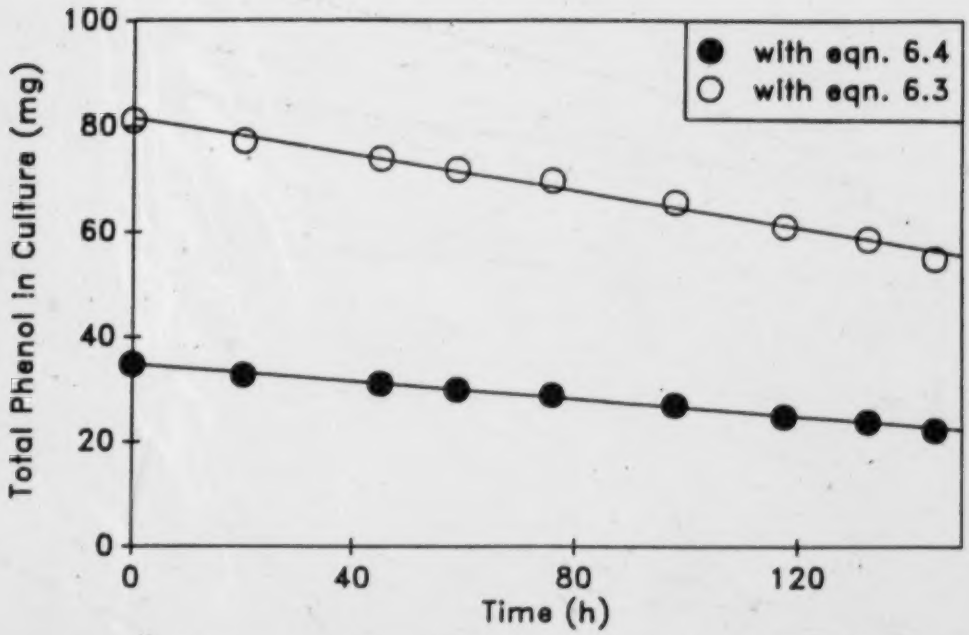


FIGURE III.6: Pre–shock load activity in GAC culture 2. Total phenol in the culture (sum of adsorbed phenol and phenol in supernatant) is on the vertical axis. Top curve is upper bound on total phenol calculated using the experimentally determined phenol adsorption isotherm for clean GAC (equation 6.3). Bottom curve is lower bound on total phenol calculated using the experimentally determined phenol adsorption isotherm for GAC from the bioreactor column (equation 6.4). Linear least–square fits to the data are shown.

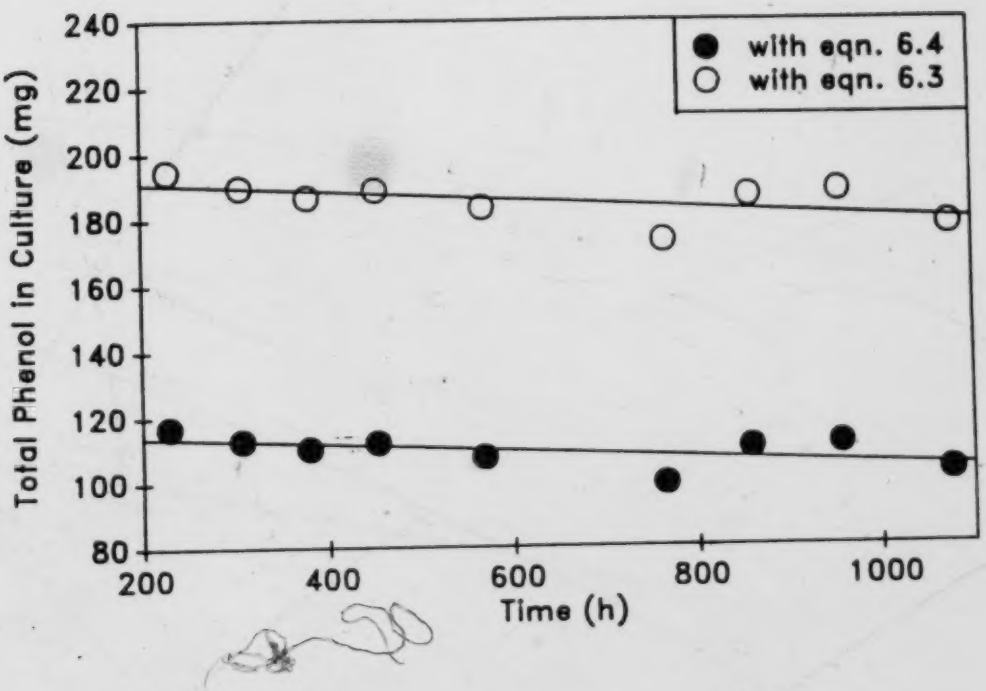


FIGURE III.7: Post–shock load activity in GAC culture 3. Total phenol in the culture (sum of adsorbed phenol and phenol in supernatant) is on the vertical axis. Top curve is upper bound In total phenol calculated using the experimentally determined phenol adsorption isotherm for clean GAC (equation 6.3). Bottom curve is lower bound on total phenol calculated using the experimentally determined phenol adsorption isotherm for GAC from the bioreactor column (equation 6.4). Linear least–square fits to the data are shown.